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PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION E

HAZARD EVALUATION:

WILDLIFE AND AQUATIC ORGANISMS

by

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Foreword

Subdivision E describes protocols which may be used to perform fish and wildlife effects testing to support the registration of pesticides under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). It is a nonregulatory companion to 40 CFR Part 158, Data Requirements for Registration. Subdivision E has been the subject of comment at a series of public meetings, the last of which occurred in July, 1982. Data requirements established by 40 CFR Part 158 are discussed in Subdivision E so that it can be read as a complete package and so that fish and wildlife testing procedures can be explained in their proper context.

Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms

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I. ORGANIZATION AND PHILOSOPHY OF SUBDIVISION E

A. Purpose.

Subdivision E provides guidelines for testing and information on data submission concerning the effects of pesticides on wildlife and aquatic organisms. Data developed according to the guidelines in this Subdivision and submitted to support the registration of a pesticide product, will be used by the Agency for determining potential hazards to nontarget birds, wild mammals, fish and aquatic invertebrates.

The tests in §§ 71-1, 71-2, 72-1, and 72-2 provide basic toxicity information which serves as a starting point for pesticide hazard assessments. These data are used principally for the following evaluations:

- To establish acute toxicity levels of the active ingredient to the test organisms;

- To compare toxicity information with measured or estimated pesticide residues in the environment to assess potential impacts to fish and wildlife;

- To provide data which determine the need for (and support the wording for) precautionary label statements to minimize the potential adverse effects to wildlife and aquatic organisms; and

- To indicate the need for further laboratory and/or field studies.

Additional tests (i.e., avian, fish, and invertebrate reproduction and life-cycle studies) are provided when basic data and environmental conditions suggest possible problems. Data from these tests are used principally:

- To estimate the potential for chronic impacts, taking into account the measured or estimated residues in the environment; and

- To determine if additional field or laboratory data are necessary to further evaluate hazards.

Simulated field and/or actual field tests are provided so that data can be developed to examine acute and chronic adverse effects on captive or monitored fish and wildlife populations in natural or near-natural environments. These data are needed only when predictions as to possible adverse effects in less extensive studies cannot be made, or when the potential for adverse effects from the use of a pesticide is likely to be high.

B. Approach.

1. Organization. The toxicity tests in Subdivision E have been grouped into two broad areas: tests for pesticidal effects on birds and mammals (§§ 71-1, -2, -3, -4, -5); and tests for pesticidal effects on fish and aquatic invertebrates (§§ 72-1, 2, -3, -4, -5, -6, and -7). Each of these groups has been further subdivided into short-term acute and subacute, reproduction, simulated field, and full field studies.

The tests are arranged in a hierarchical or tier system which progresses from the basic laboratory tests to the applied field studies. The results of each tier of tests should be evaluated to determine the potential of the pesticide to cause adverse effects and to determine whether further testing should be considered. It is expected that each sequential test will be performed far less often than the preceding test. Figures 1, 2 and 3 of § 70-1(b) illustrate the tier testing system for avian wildlife, wild mammals, and aquatic organisms, respectively.

2. Content of paragraphs within sections. Within Subdivision E, each section provides guidance for a particular test or group of related tests, and contains the following provisions:

- A provision refering to 40 CFR § 158.145 to determine when the data are required, and containing additional guidance on when the data are required to support the registration of the manufacturing-use product or formulated product or both;

- Provisions outlining test standards that should be complied with in conducting the studies;

- Provisions outlining standards for reporting and evaluating test data; and

- Examples of acceptable protocols, references to published documents and technical journals, and other aids that will help in planning and conducting toxicity tests.

3. Reporting standards. Section 70-4 "Reporting and Evaluation of Data" provides guidance on the general reporting and evaluation standards for this subdivision. In addition, each section contains a paragraph entitled "Reporting and evaluation of data" which sets forth the Agency's particular standards that apply to each test.

4. Acceptable protocols and references. Each section includes paragraphs which generally provide the following kinds of information:

- Examples of acceptable test protocols;

- References that may provide useful background information in developing acceptable protocols; and

- References that outline useful statistical procedures for handling data.

The examples of acceptable protocols are either those which are not available in any other published form or which are of widespread interest because they provide examples of tests which would be routinely performed for a significant number of products. The Agency believes that publishing the acceptable protocols and references with each section is the most efficient way of providing this useful information to all potential users.

References to standard practices established by the American Society for Testing and Materials for conducting freshwater fish acute toxicity studies, freshwater aquatic invertebrate acute toxicity studies, and marine mollusc shell deposition and embryolarval toxicity studies, appear in the appropriate aquatic test sections as examples of acceptable protocols. Various subcommittees within ASTM are currently preparing standard practices for other studies required by this subdivision, such as the avian single-dose oral LD50, avian reproduction, and aquatic accumulation studies. When these standard practices are completed, the Agency will review them, and consider including them in appropriate sections of this subdivision as acceptable test protocols.

5. Relation of Subdivision E to other subdivisions. Subdivision E provides standards for testing and data submission concerning the effects of pesticides on nontarget birds, mammals, fish, and aquatic invertebrates. The data developed according to Subdivision E and required by 40 CFR § 158.145 represent only a portion of the data considered in evaluating hazards to nontarget organisms. The Agency also routinely examines other information submitted in response to other sections of 40 CFR Part 158 requirements. In evaluating the potential hazards, the Agency also considers data developed according to Subdivision D, entitled "Chemistry Product Chemistry," Subdivision F, entitled "Hazard Evaluation: Humans and Domestic Animals," and Subdivision N entitled "Chemistry Environmental Fate."

Data required in 40 CFR § 158.135 and developed according to Subdivision F are normally adequate to indicate hazard to wild mammals. Under certain conditions, these data are not sufficient to assess the potential hazards to wild mammals and further tests should be conducted. Section 71-3 provides examples of circumstances when additional tests and data on wild mammal toxicity may be needed.

Together, the data required by 40 CFR Part 158 are used by the Administrator to make the determination, required by FIFRA sec. 3(c)(5), whether a pesticide "...will perform its intended function without unreasonable adverse effects on the environment" and thus whether the pesticide may be registered. The data are also used by the Administrator to make the "incremental risk" finding required by FIFRA sec. 3(c)(7) (conditional registration).

Subdivision E currently does not provide testing guidelines to address the effects of pesticides on nontarget amphibians and reptiles. At the present time, the Agency assesses hazards to these nontarget organisms from the use of pesticides on a case-by-case basis, using all available and appropriate data. The Agency is currently gathering literature on the effects of pesticides on amphibians and reptiles, and on the appropriate test methods needed to measure these effects. After a review of the available literature, the Agency may determine:

- That the data required by 40 CFR Part 158.145 and developed according to Subdivision E of the guidelines are sufficient to determine hazards to nontarget amphibians and reptiles; or

- That additional data are needed in order to determine hazards to these nontarget organisms.

The latter would necessitate the establishment of data requirements in 40 CFR § 158.145 and the development of guidelines for testing and data submission concerning the effects of pesticides on nontarget amphibians and reptiles.

II. MAJOR ISSUES

Public comments and Agency review of the 1978 proposed Subpart E guidelines identified a number of major issues which are presented in this discussion. Many comments covering both major and minor points were adopted by the Agency, and corresponding changes in the guidelines were made. Discussions of the major issues brought up by public comments are presented in the following paragraphs.

A. Data Requirements for Manufacturing-Use Products.

In the Preamble to the 1978 proposed Guidelines, EPA asked for public comment on the question as to whether or not the data requirements of this subdivision should be extended to manufacturing-use products. After serious consideration of this issue, the Agency has concluded that extending the data requirements to such products is appropriate. The Agency was influenced by the views of commenters on this issue who generally favored a data submission requirement

which makes the basic manufacturer of an active ingredient responsible for providing most of the effects data.

Therefore, 40 CFR § 158.50, entitled "Formulators' Exemption" requires a registrant of a manufacturing-use product to submit (or cite) any data pertaining to the safety of an active ingredient in its product if the same data are required to support the registration of an end-use product that could legally be produced from the registrant's manufacturing-use products. (An end-use product is a pesticide product bearing label directions for immediate end-use as a pesticide.) Section 158.50 also provides that such data must be submitted by an applicant for registration of the end-use product, except that the producer of the end-use product will generally not have to submit or cite data pertaining to registered products which the end-use producer purchases and uses to formulate the end-use product. This decision reflects the Agency's expectation that manufacturing-use product registrants will be the major source of registration data, and that end-use product formulators will, in most cases, need to suply much less data. This decision is consistent with the provisions of, and Congressional intent behind, sec. 3(c)(2)(D) of FIFRA, which provides that:

> No applicant for registration of a pesticide who proposes to purchase a registered pesticide from another producer in order to formulate such purchased pesticide into an end-use product shall be required to --
> (i) submit or cite data pertaining to the safety of such purchased product; or
> (ii) offer to pay reasonable compensation otherwise required [§ 3(c)(1)(D) of FIFRA] for use of any such data.

Implicit in sec. 3(c)(2)(D) is Congress' expectation that it would be the registrant of the manufacturing-use product who would provide significant amounts of data pertaining to the safety of its product. (See, e.g., Sen. Rep. No. 334, 95th Cong., 1st Sess., pp. 8-9.)

Moreover, if data requirements were imposed solely on registrants of end-use products, sec. 3(c)(2)(D) might be read to prevent the Agency from obtaining data on the grounds that the data pertain to the safety of a purchased product.

B. Degree of Specificity in the Guidelines.

In responding to the proposed version of the guidelines, commenters argued both that the guidelines were too specific and that they were too general. The Agency has sought to strike a balance between

these concerns. The Agency has rejected a step-by-step or "cookbook" description of test procedures because it recognized that any specific detailed method may not be suitable for the evaluation of all pesticides, and that new test procedures are constantly being developed. For example, the Agency believes that testing guidelines should have the flexibility to take into account differences in test equipment, personnel, animals at various laboratories, and characteristics of the pesticide actually being tested. Consequently, the Agency has sought to establish test standards which possess the specificity necessary for data comparison, but retain the flexibility to accommodate the other considerations noted above.

C. Reporting of Data.

Several commenters suggested that not all the information listed under proposed § 163.70-1(c) Reporting of data needs to be submitted with each report. They recommended that information such as the physical and chemical properties of the test substance, assay methods, and identity of the test substance should be submitted to the Agency only once with each application. The Agency agrees, and does not request duplicative submission of data. Therefore, references to data furnished in response to the requirements of 40 CFR Part 158 will be accepted, provided that the complete text of the data is actually supplied elsewhere in a submittal and is properly marked or headlined for reasonable accessibility.

EPA has also revised the general reporting standards in Subdivision E in response to suggestions that the standards which do not apply to all or most of the tests be deleted from this section. Accordingly, the Agency has revised and relocated these reporting standards. They now appear in the sections prescribing reporting requirements for specific tests. A few reporting standards that apply to a majority, but perhaps not all, of the tests in Subdivision E have been retained in § 70-4 with appropriate qualifying statements. For example, the standard proposed in § 163.70-1(c)(7)(iii) now reads [at § 70-4(c)(7)(ii)] "Calculation of the LD50, LC50, or EC50, and the 95 percent confidence interval, when sufficient doses and test organisms are used to establish a dose-response line." Proposed § 163.70-1(c)(2) Environmental conditions has also been revised [at § 70-4(c)(6)] so that the data reporting standards for tests conducted with mammals or birds and those conducted with fish or aquatic invertebrates now appear in separate paragraphs.

D. Test Substance.

There was considerable discussion of the Agency's choice of the test substance that should be used in the proposed Subpart E tests. Some commenters argued that only the formulated product should be tested because isolation of the active ingredient is not always feasible. They also argued that neither the manufacturing-use product nor the active ingredient in technical form poses an environmental hazard to wildlife or aquatic organisms when it is used or distributed in commerce. Other commenters, however, felt that testing of the technical grade of each active ingredient would be sufficient because the toxicity of a pesticide product depends almost exclusively on the concentration of the active ingredient in the product. They felt that the "inert" ingredients in a pesticide (those which do not cause the intended pesticidal effect) usually have no independent toxic effect on wildlife or aquatic organisms.

The Agency has considered both sides of the issue, and has determined that short-term acute toxicity studies should be performed on the active ingredient of the pesticide. Therefore, the fish and wildlife guidelines now provide that all tests except simulated and actual field studies (§§ 71-5, 72-6 and -7) should be conducted with the technical grade of active ingredient. (The simulated and actual field studies should be conducted with the end-use formulation.) This position is based on the Agency's conclusion that, in most cases, the toxicity of an end-use formulation is directly proportional to the toxicity of the active ingredients in the product and their amounts. The Agency is aware that the acute toxicity of the technical grade of an active ingredient can differ significantly from that of the end-use formulation containing that active ingredient. The Agency also recognizes that the various inerts (e.g., emulsifiers, surfactants, binders, etc.) in an end-use formulated product may affect the toxicity of the active ingredient. Nevertheless, the Agency believes that short-term acute toxicity tests conducted with the active ingredient will provide adequate information for identifying end-use products which may potentially pose risks to wildlife or aquatic organisms. These risks can be further evaluated in additional studies, including pen or field tests, if necessary. In addition, short-term acute toxicity tests conducted with the technical grade of the active ingredient will have less of an economic impact than short-term acute toxicity tests conducted with each end-use formulated product.

There will be circumstances where an exception to the above rule is appropriate. Testing end-use formulations, certain inert ingredients, or other product components of concern, in short-term tests, will be required according to 40 CFR § 158.75(b) on a non-routine as-needed basis. Past experience shows that data from these tests conducted with end-use products are only required in unusual cases. Testing with end-use formulations in short-term

aquatic tests is required by 40 CFR § 158.145 only under the following situations:

- When the end-use product is expected to be introduced directly into the aquatic environment when used as directed;

- When an ingredient in the end-use product other than the active ingredient is expected to enhance the toxicity of the active ingredient; or

- When a specified EC50 of the technical grade of the active ingredient approximates the expected residue level in the aquatic environment.

The Agency is aware that a single registration of pesticide products sometimes encompasses multiple formulations (i.e., same active ingredient at same concentration but varied inert ingredient components and concentrations). Agency concern about multiple formulations is confined principally to formulation testing in §§ 71-5 and 72-7. Under these two studies, the Agency permits test results to be obtained from testing a representative formulation for the registration under consideration. This is because, in most instances, the overriding adverse effect would be due to the active ingredient, which is the ingredient of principal concern. The Agency according to 40 CFR § 158.75(b) may, on a case-by-case basis, require testing of more than one of the multiple formulations under a single registration; but this will not occur often. In these instances, the concern would be with evaluation of possible adverse effects that may be attributed to the various inert ingredients that would differ from formulation to formulation under the same registration.

E. Endangered Species.

Several commenters argued that the guideline standards which recommend against the conduct of pen and field studies in areas containing, or suspected to contain, threatened or endangered plants or animals [current §§ 71-5(b)(4) and 72-7(b)(4)], are excessively restrictive and could place unnecessary and unrealistic limitations on field testing. One commenter stated that large areas of the U.S. could not be used if this policy were strictly enforced, especially for endangered animals that are mobile, far-ranging, or migratory. Another commenter would like to see this guideline standard deleted because it pertains to the preservation of wildlife rather than the evaluation of chemicals.

With regard to endangered species that are mobile, far-ranging, or migratory, the Agency recommends that field tests not be conducted in areas determined to be "critical habitat" by the Office of Endangered Species of the U.S. Fish and Wildlife Service

or in areas known, or suspected, to contain individuals of these endangered species.

F. Selection of Avian Species.

Several commenters suggested using avian species other than mallard, pheasant, and bobwhite quail for various toxicity tests. The test species that were suggested included domestic chickens and ducks, Japanese quail, and songbirds. Some commenters felt that there were no practical differences between Japanese quail and bobwhite quail for pesticide hazard evaluation purposes. They believed, moreover, that Japanese quail is as sensitive to toxic effects as the bobwhite, and, like the bobwhite, there is an existing data base on the effects of pesticides on this species. Other commenters felt that songbirds were more sensitive and more representative of the North American avifauna than the recommended species and, therefore, should be used. Finally, according to some commenters, domestic ducks and chickens have not been demonstrated in the published literature to be less sensitive than the preferred species, and therefore ducks and chickens should be equally acceptable as test species.

In response to the very emphatic and numerous comments on this topic, the Agency's determinations and rationales pertaining to preferred species for use in avian tests follow in the next several paragraphs. Generally, the species named in the 1978 proposal for the avian test were retained for the current guidelines.

Ideally, the evaluation of the toxicity and hazards of a pesticide to wildlife would be made under all field conditions in which the pesticide may be used, and would be made using as many species as would likely be exposed. However, the number of species to be evaluated, the number of random, uncontrollable variables, and the size and complexity of some application sites, combine to make such testing completely impractical on a routine basis. Thus, for purposes of hazard evaluation, it is necessary to limit investigations to those species which will yield the most useful information at a cost, in time and money, that is not prohibitive.

Recognizing, therefore, that it would be impossible to test every potentially exposed species, EPA has recommended one or more species which would provide data most useful for hazard evaluation and consistent comparison among chemicals. The following criteria, which are not listed in order of importance, have been considered in choosing species to be tested:

- (A) The test species should be one which has demonstrated sensitivity to the effects produced by known toxic chemicals; that is, it should be a species about which the Agency possesses

substantial historical information on the dose/response of the test organism to a variety of pesticides. With such information, EPA can be relatively confident that the species will also be sensitive to toxic effects caused by a previously unevaluated chemical. In addition, such baseline data allow EPA to compare the toxicity of different pesticides, the results obtained in different test facilities, and the results obtained in the same test facility over a period of time. The latter two comparisons are one way in which EPA can check the quality of the data which it receives. The first comparison is useful in hazard evaluation.

- (B) The test species should be ecologically significant, i.e., one that occurs naturally in large numbers and in widespread habitats. Since hazard evaluation is made according to the use pattern, the most appropriate test animals will be those that occur naturally in the ecological communities associated with the use pattern. If only a limited number of test species are to be recommended, then a substantial benefit is gained by testing a species that is abundant and widely distributed, because birds from that species are likely to be exposed to pesticides from a variety of use patterns. A number of species might be suitable simply on the basis of sensitivity, but with widely distributed species there is an additional advantage in that a more direct extrapolation from laboratory tests to field situations can be made.

- (C) For the purposes of this Subdivision, it is desirable to use a test species which is aesthetically or economically valuable to man. This is particularly appropriate, considering that many Agency decisions are based on risk-benefit analyses.

- (D) Test organisms should be readily available for test purposes. Moreover, they should not be endangered or threatened species, they should be amenable to captivity, and they should be economical to maintain in a laboratory environment. For a test species to meet these criteria, relatively extensive information about the nutritional, habitat, and behavioral characteristics of the natural population should be known. The source and history, including origin of breeding stock, genetic composition, and history of disease should be accessible for documentation.

- (E) The characteristics of test organisms should be appropriate to the types of tests being conducted. They should demonstrate observable effects within a reasonable period of time. For example, they should have a life cycle short enough to accommodate reasonably short (1-2 years) chronic and reproductive tests. They should also be appropriate for acute, dietary, reproductive, and simulated field tests so that the comparisons of the various tests can be made within the species. They should be amenable to an experimental design that can be statistically analyzed.

For example, birds that lay only two eggs per year are generally not appropriate for reproduction tests. Finally, it is desirable that the test organisms not be overly susceptible to disease, parasites, and handling.

Not surprisingly, no single species completely satisfies all of the above criteria for every kind of test. The available literature dealing with the toxicity of pesticides to avian species indicates that, for the majority of pesticides tested, mallard and bobwhite are as sensitive or more sensitive than Japanese quail and other domestic birds. If sensitivity were the only criterion for the selection of test species, the choice between the species would be difficult to make. However, many other factors (as mentioned above) influence the selection of acceptable test species.

After much debate and after considering the criteria presented above, the Agency believes that, at least for the present, allowing the use of Japanese quail for toxicity tests is not appropriate. The primary advantage to using Japanese quail is that they are easily and economically maintained and bred in the laboratory. However, the Japanese quail is generally no more sensitive than bobwhite quail and has a smaller toxicity data base than bobwhite quail. Furthermore, the Japanese quail has not become a part of any North American ecological community, and consequently we know little about how it would behave if used in field studies. Therefore, some species other than Japanese quail would probably be required for higher tier studies, and EPA would lose the advantages of comparing data from different kinds of studies on the same species. The same observations apply similarly to the domestic duck and chicken. Accordingly, the Agency believes that these three species are not as suitable as the ones selected for these guidelines. However, it will review the question over the next several years as new data become available.

The Agency agrees that songbirds may often be more sensitive to pesticides than the species required by the guidelines. The Agency also recognizes that many of the nontarget species exposed to a pesticide may be songbirds and, therefore, they may be more representative of the exposed avian species than the species required by these guidelines. However, there are no data which show that results from testing with a species of songbird are more useful than bobwhite or mallard for predicting the effects of a pesticide on a large number of bird species. In the future, the Agency may include songbirds in its test scheme. However, the Agency recognizes that the feasibility of using songbirds for testing on a regular basis has not been demonstrated except possibly for the acute oral toxicity test.

G. Age of Birds.

Several commenters recommended changes in the age range proposed in the avian testing standards of §§ 163.71-1(b)(3), 163.71-2(b)(4), and 163.71-4(b)(4). Commenters suggested that younger birds, 10 to 17 days old, should be used in the avian single dose oral test because they are more sensitive, more uniform in response, more available, less expensive, and easier to house and handle. They felt that use of the same age birds for both acute and dietary tests would provide greater consistency and comparability. Concerning the avian dietary test, commenters felt that there was too much flexibility allowed in the current age requirement of 10 to 17 days. Some recommended ages were: at the time of yolk sac absorption, 10-16 days for quail, and 5-11 days for mallards. The latter was suggested by a commenter because he stated that older mallard ducks can refuse to eat during the 5-day period in which they would be exposed to treated food and therefore survive. Thus, results from such tests could indicate no hazard when the birds were really not exposed to the treated food during the test. Other commenters recommended that older birds which are still within the birds' normal reproduction age range should be allowed in the avian reproduction test, because first year birds (current standard age) are less productive and more variable in egg production.

In response to the numerous comments on this topic, the Agency's determinations and rationales pertaining to the age of birds used in avian tests follow in the next several paragraphs. Except for the avian dietary tests, the age of birds provided in the 1978 proposal were retained for the current guidelines.

EPA has considered several factors including sensitivity in setting the age of birds for use in avian acute oral and dietary tests. First, there is a substantial data base for mallards and quail that are 16 weeks or older, and also for those that are 10 to 17 days of age. There is a growing data base for mallards that are between 5 and 10 days old. Outside of these ages, the data base is meager. Second, EPA has reviewed data from sufficient numbers of tests to show that birds within the proposed age ranges normally provide adequate uniformity of response to determine the toxicity level. Finally, the Agency intended that all of the birds in any test should be the same age, and has clarified the wording of the guidelines in this respect. This is especially crucial in the dietary study which uses juvenile birds, where a range of several days in the age of the birds could result in excessive variation in the results of that test. Thus, the guidelines recommend that bobwhite be between 10 and 14 days old and mallards should be between 5 and 10 days old. In both cases all birds in a study should be from the same hatch.

With regard to the avian reproduction test, the guidelines continue to recommend birds approaching their first breeding season for the following reasons:

- Because the Agency can be reasonably assured that the birds have not been previously exposed to pesticides as adults;

- Because variability is markedly reduced when all test birds are of the same age;

- Because the age of immature birds approaching their first breeding season can be determined more accurately than the age of adult birds; and

- Because first-year birds would be of lower cost than second or third-year birds.

H. Avian Test Doses and Concentrations - Criteria for LD50 and LC50 Determinations.

Two sections of the proposed guidelines, § 163.71-1 "Avian single-dose oral LD50 test" and § 163.71-2 "Avian dietary LC50 test," provided that substances not particularly toxic to birds need not be tested as thoroughly as more toxic chemicals. Specifically, these sections stated that the applicant may submit data showing that the avian LD50 is greater than 2,000 mg/kg, or that the avian LC50 is greater than 5,000 ppm, instead of determining precise LD50 or LC50 values. Several commenters wanted to know the Agency's rationale for selection of these values.

The Agency provided these "cut off" levels as an option to the determination of precise LD50 and LC50 values for relatively innocuous compounds. These values (2,000 mg/kg and 5,000 ppm) are arbitrary. However, the Agency believes that few (if any) pesticides will be applied at a label rate that will result in residues equal to or greater than these values. In addition, these "cut off" levels have a history of use in the literature on avian toxicology, as evidenced by:

- (A) Tucker, R.K., and D.G. Crabtree. 1970. Handbook of Toxicity of Pesticides to Wildlife. Fish and Wildlife Service Resource Publication 84. U.S. Dept. Interior, Washington, D.C., 131 pp.; and

- (B) Hill, E.F., R.G. Heath, J.W. Spann, and J.D. Williams. 1975. Lethal dietary toxicities of environmental pollutants to birds. U.S. Fish and Wildlife Service Special Scientific Report - Wildlife No. 191. U.S. Dept. Interior, Washington, D.C. 61 pp.

I. Avian Single-Dose Oral LD50 (§ 71-1).

A number of commenters questioned the need for the avian single-dose oral LD50 test (proposed § 163.71-1). Various reasons were given by the different commenters:

- The acute oral test is artificial and not representative of field situations;

- The acute oral test often provides a misleading picture of hazards through underestimation of the toxicity of accumulative chemicals or overestimation of the toxicity of easily degraded chemicals;

- Data from the dietary LC50 tests provide a reliable basis for assessing potential hazards, even for granular formulations; and

- There are too many bird tests in the guidelines.

Finally, another commenter suggested that performing this test is only appropriate if the dietary LC50 is less than 500 ppm or if other data indicate an unusual hazard.

The Agency recognizes that the avian acute oral test does not represent actual exposure in field situations, except in the case of granular formulations, and baits. The consumption of pesticide granules and baits by birds is an exposure that is very similar to the exposure presented by the laboratory LD50 test. The Agency contends that either the acute oral test or the dietary test, used alone, may be misleading in evaluating a pesticide that has cumulative effects, or one that is easily degraded. One or the other, however, is likely to represent acute toxicity, and the two in concert should adequately define the potential hazard to birds.

The Agency rejects the contention that dietary tests are reliable indicators for granular formulations, particularly when one or a few granules exceed the avian LD50 level. Many N-methyl carbamates, for example, are formulated into granules and are much more toxic to birds in acute oral doses than in dietary doses. Published data and data in Agency files indicate substantial disparity between acute and dietary toxicities of carbamate pesticides. This disparity, which also exists for a few other pesticides, is great enough that reliance on the dietary toxicity alone could result in unsound hazard assessments.

The Agency also rejects the contention that the guidelines contain an excessive number of avian tests. The two avian tests that are routinely required by 40 CFR § 158.145 are relatively inexpensive and easy to perform, and they are essential to assess hazards

to various avian species. The costs of these tests in 1982 are estimated to be $1800 for the avian single-dose oral LD50 test, and $3600 for both avian dietary LC50 tests.

The Agency uses data from the avian acute oral LD50 test to establish the acute toxicity level of a pesticide to avian species. The test also provides additional information that is useful for determining hazards of a pesticide to birds. For example, symptomology is used to establish the toxic action of the pesticide to avian species. The slope of the avian LD50 dose-response line provides information on safety factors for acute effects; a gradual slope requires a higher safety factor for acute effects than does a steep slope.

J. Mammalian Acute Toxicity (§ 71-3).

Based on the number and nature of comments received in regard to proposed § 163.71-3 (formerly "Mammalian Acute Toxicity," now "Wild Mammal Toxicity"), the Agency has concluded that this section needed revision to eliminate misunderstandings regarding the guidance when such data would be required and what test procedures would be acceptable.

The guidelines now expressly state that data from this test are not routinely required by 40 CFR § 158.145, since mammalian data required by 40 CFR § 158.135 and developed according to Subdivision F (Hazard Evaluation: Humans and Domestic Animals) are normally sufficient for assessing the hazards to wild mammals. In addition, the "When required" paragraph has been revised to reduce the unintended emphasis on ruminants. The testing standards and protocols have also been modified and expanded in order to address the diversity of situations in which additional testing might be necessary and to provide more guidance for applicants in developing methods to address specific situations. Additional minor changes have been made in the standards for reporting and evaluation of data.

Although there are well-developed and tested methods for determining the toxicity to laboratory animals, toxicity testing has not been extensively performed with wild mammals. The Agency welcomes suggestions for protocols that are based on reproducible experimental data.

K. Avian Reproduction Test (§ 71-4)

Several years ago the Agency recognized that pesticides were causing reproductive impairment in some bird populations. Consequently, in § 162.82 (c)(2)(ii)(A) of the 1975 proposed guidelines and in § 163.71-4 of the 1978 guidelines, the Agency decided to provide avian reproduction tests for chemicals which had the potential to affect reproduction in birds. The design of these tests, as with most test protocols, is dynamic. The Agency is constantly striving to update and improve these protocols.

In the past, these guidelines have recommended a testing design which placed groups of birds in each pen (i.e., mallards: 2 males, 5 females; and bobwhite: 1 male with 2 females). With this arrangement the number of replicates (pens) needed for each treatment was 5 and 12 for mallard and bobwhite, respectively. At the 1980 FIFRA Scientific Advisory Panel meeting, the Agency concurred with the option of pairs testing to accomodate various segments of the scientific community who support such a design. Now that data are available, the Agency has analyzed the efficiencies of both testing designs. The group design provided sufficient sensitivity to detect a statistically significant reproductive impairment of 20% or more using the recommended number of replicates. For the pairs testing to achieve this sensitivity, more than 12 replicates are necessary; calculations have indicated that as many as 25 replicates may be necessary.

Based on our analysis, the Agency continues to recommend the group-pen design. This is consistant with the previous proposed guidelines. A registrant, in consultation with a testing facililty, may wish to use the pair-test design. In this case an attempt should be made to design a test which provides sufficient sensitivity to detect statistically significant reproductive impairment of 20% or more. A reference for calculating the number of replicates needed for a particular level of sensitivity is now included in the guidelines.

This change will result in a more consistent determination of reproductive impairment, regardless of test design. There will be no increase in cost if the group-pen design is selected; however, a moderate increase in cost for the pair-pen test design may be incurred due to the increased number of replicates.

L. Small Pen Field Test (§ 71-5) - Protocol.

Commenters of proposed § 163.71-5 recommended deleting the small pen field test, citing numerous deficiencies in the protocol for such tests. Among the problems noted were:

- That such tests do not accurately predict the hazard to wild birds whose behavior differs from that of domestic birds;

- That the pen must be moved frequently so that the birds can be exposed to the pesticide and adequate food sources; and

- That the birds are vulnerable to attack from predators.

The Agency recognizes the limitations in the design of small pen tests referred to above, some of which were mentioned in the preamble to the 1978 proposed guidelines. In that preamble, the Agency also requested suggestions on how to improve the test design to avoid these problems, but received no useful suggestions from the public. The Agency again welcomes suggestions that can be supported by data, literature, or specific rationales.

Until a better small pen test is developed and tested for validity, feasibility, and comparability, the Agency has deleted the existing small pen protocol from the guidelines. The existing large pen protocol, with some modifications, will be used in its place. Data produced by using the large pen test have proven to be more useful to the Agency than those from small pen tests.

M. Actual Field Test (§ 71-5).

Commenters have stated that the actual field test for mammals and birds is too comprehensive, complex, expensive, and time-consuming, and therefore such tests should be eliminated. They have also pointed out problems with specific aspects of the protocol in proposed § 163.71-5. The Agency fully recognizes the complexity and cost of a full-scale field test, and consequently, the Agency provides this test only to evaluate those products which pose significant risks to nontarget birds or mammals. The studies conducted in previous tiers are designed to yield specific information which forms the basic foundation of a hazard assessment. Because of their design, however, the lower tier studies cannot always be used to adequately predict the effects of the pesticide in real use situations. Therefore, it may become necessary to perform the actual field test when the previous laboratory or cage studies demonstrate toxicity at normal use rates or indicate potential long-term adverse effects. The actual field study results would then either support earlier indications and alter plans for certain uses or for prospects of obtaining a registration, or they would disprove the potential hazards evinced in the earlier tests and thus maintain proposed uses and improve prospects of obtaining product registration.

EPA notes that each actual field test should be specifically tailored to address the concerns that necessitate it. In developing a test methodology, applicants are advised to consult with appropriate EPA scientists to assure that the information obtained is pertinent and sufficient to satisfy the Agency's hazard evaluation requirements.

In view of the diversity of potential study areas, (e.g., forests, rangelands, rights-of-way, wetlands; and major croplands such as cotton, wheat, corn, etc.) field studies should be designed on a case-by-case basis. Consequently, the previously published protocol has been deleted. In its place are a number of selected references that can be used as guidance for developing actual field studies.

N. Aquatic Test Concentrations - Criteria for LC50 Determination.

Several sections of the guidelines for acute toxicity testing with aquatic organisms provide that substances which are not particularly toxic need not be tested as thoroughly as more toxic chemicals. Specifically, these sections state that the applicant may conduct a range-finding study, and if the LC50 is greater than 300 mg/l or is greater than 300,000 times the expected environmental concentration, the determination of a precise LC50 value is not necessary. [See §§ 72-1(b), -2(b), -3(b).] Several commenters suggested that these "cut-off" levels are too high.

In light of public response and the Agency's experience in working with the previously established levels, the Agency has decided to reduce the levels. The intent of the "cut-off" levels is to preclude the need for definitive acute tests when the range-finding tests indicate the chemical/pesticide to be relatively non-toxic. After using the previous "cut-off" levels for several years, the Agency has not had to request additional acute studies. Accordingly, the Agency has decided to reduce the levels to a concentration of 100,000 times the EEC or 100 mg/l. If, after using the new levels, the Agency finds they do not provide adequate data for hazard assessment, additional changes will be made. Note that the standard for testing 30 organisms at either of these criteria with less than 50 percent mortality remains unchanged.

O. Selection of species for freshwater fish acute toxicity studies.

Considerable public comment focused on the number and choice of test species for freshwater fish acute toxicity studies. Basically, these comments favored use of only one species, rather than the two

fish species required by the proposed guidelines, § 163.72-1. In addition, the commenters suggested a number of factors which should be considered in selecting the species to be tested, such as the most sensitive species, the species most likely to be exposed under normal use conditions, or the species which would be used if higher tier tests are required. Also, one commenter recommended the fathead minnow as a test species in the fish acute toxicity study because the fathead is also used in the fish life-cycle study. In the past, industry representatives and other concerned individuals have asked for a more precise definition of the terms "coldwater fish" and "warmwater fish." The guidelines provided for the testing of fish from both categories, and since these categories were loosely defined by examples, a more precise definition was needed. The characteristics of the fish families Centrarchidae and Salmonidae have been used to exemplify a warmwater and coldwater fish, respectively.

The Agency has defined coldwater fish as those fish which normally spawn in the fall and winter months in water colder than 60°F (15°C). Warmwater fish are defined as those fish which normally spawn in the summer months in temperatures greater than 60°F. Examples, as previously stated, are the Centrarchids and Salmonids for warmwater and coldwater fish. The preferred warmwater species is the bluegill sunfish (Lepomis macrochirus) and the preferred coldwater fish is the rainbow trout (Salmo gairdneri). Data from two species are used to indicate the extent of variation in sensitivity between the different species. In addition, by testing two species, the Agency will have data available on fish representative of a much larger geographical range than if only one species were tested. These considerations outweigh the small additional costs associated with testing a second species. Finally, while the Agency would not reject data from tests using the fathead minnow, it is not as sensitive to most insecticides as the bluegill or rainbow trout, and thus is not generally preferred for this study.

P. Marine Toxicity Tests.

Commenters objected to the testing provided in proposed § 163.72-3 for developing data on the acute toxicity of a pesticide to marine and estuarine organisms. They stated that this testing is not needed every time a pesticide is applied directly to marine or estuarine environments. They argued that EPA should consider the concentration of a pesticide in these environments resulting from actual use before determining the need for this testing. For example, they suggested that if the estimated environmental concentration of a pesticide is less than the LC50 or EC50 of that pesticide for freshwater organisms, the data would not be needed.

Commenters also questioned the Agency's need for data on the toxicity of a pesticide to marine species in light of newly-published literature showing a correlation between toxicity data from tests with freshwater fish (rainbow trout) and tests with saltwater fish. Based on this relationship, the commenters suggested that the data from marine studies is not needed if a high concentration of a pesticide produced no effect in a test using freshwater fish.

The Agency is firmly convinced that it needs data on the acute toxicity of a pesticide to estuarine and marine organisms. With it, we evaluate the hazard of that pesticide when it is applied to a marine or estuarine site. Direct applications to these environments are made for the purpose of reducing the population of one or more pest organisms existing there. Thus, there is every reason to expect that the pesticide will be present in quantities which may be toxic to nontarget organisms as well.

The Agency rejects the suggestion to use data from freshwater fish toxicity studies to predict the hazards to saltwater fish. The predictive equation that has been developed based on the correlation between known rainbow trout LC50 values and known saltwater fish LC50 values for a number of chemicals cannot be used by the Agency. The 95 percent confidence limits of the predicted saltwater fish LC50 values are equal to $\pm$ 1.3, or 2.6 orders of magnitude. Saltwater fish LC50 values with confidence limits of this magnitude do not permit EPA to judge whether a particular pesticide will pose unacceptable risks when it enters the marine environment.

SUBDIVISION E - HAZARD EVALUATION: WILDLIFE AND AQUATIC ORGANISMS

Series 70: GENERAL INFORMATION AND REQUIREMENTS

§ 70-1 General information.

(a) Scope and purpose. This subdivision provides guidelines for testing and data submission concerning the effects of pesticides on nontarget birds, wild mammals, fish, and aquatic invertebrates. Each section contains standards for acceptable testing, guidance on evaluation and reporting of data, examples of acceptable testing protacols, and further guidance on when data are required. The data produced by following these guidelines will be used by the Agency for determining potential hazards to nontarget organisms resulting both from direct and indirect exposure to pesticides.

(b) Organization. (1) Categories of tests by organism group. This subdivision contains two broad categories of tests:

(i) Tests for pesticidal effects on birds and mammals (§§ 71-1, -2, -3, -4, and -5); and

(ii) Tests for pesticidal effects on fish and aquatic invertebrates (§§ 72-1, -2, -3, -4, -5, -6, and -7).

(2) Tier tests. (i) The Tier 1 tests within each of the categories named above, document the acute and subacute pesticidal effects on birds, mammals, fish and aquatic invertebrates. These tests are required by 40 CFR § 158.145 to support the registration of most pesticide products. Tier 1 tests on birds and mammals appear in §§ 71-1 and 71-2. Tier 1 tests on fish and aquatic invertebrates appear in §§ 72-1 and 72-2.

(ii) The "When required" paragraphs in §§ 71-3, -4, and -5 and 72-3, -4, -5, -6, and -7 refer to 40 CFR § 158.145 and provide further guidance as to when data, in addition to Tier 1 test results, will be required to support the registration of a pesticide product.

(iii) Figures 1, 2 and 3 (below) illustrate the tier testing system for avian wildlife, wild mammals, and aquatic organisms, respectively. These figures, however, do not constitute requirements. The applicant should refer 40 CFR Part 158 for the actual requirements.

Figure 1. Avian Testing Flow Chart.

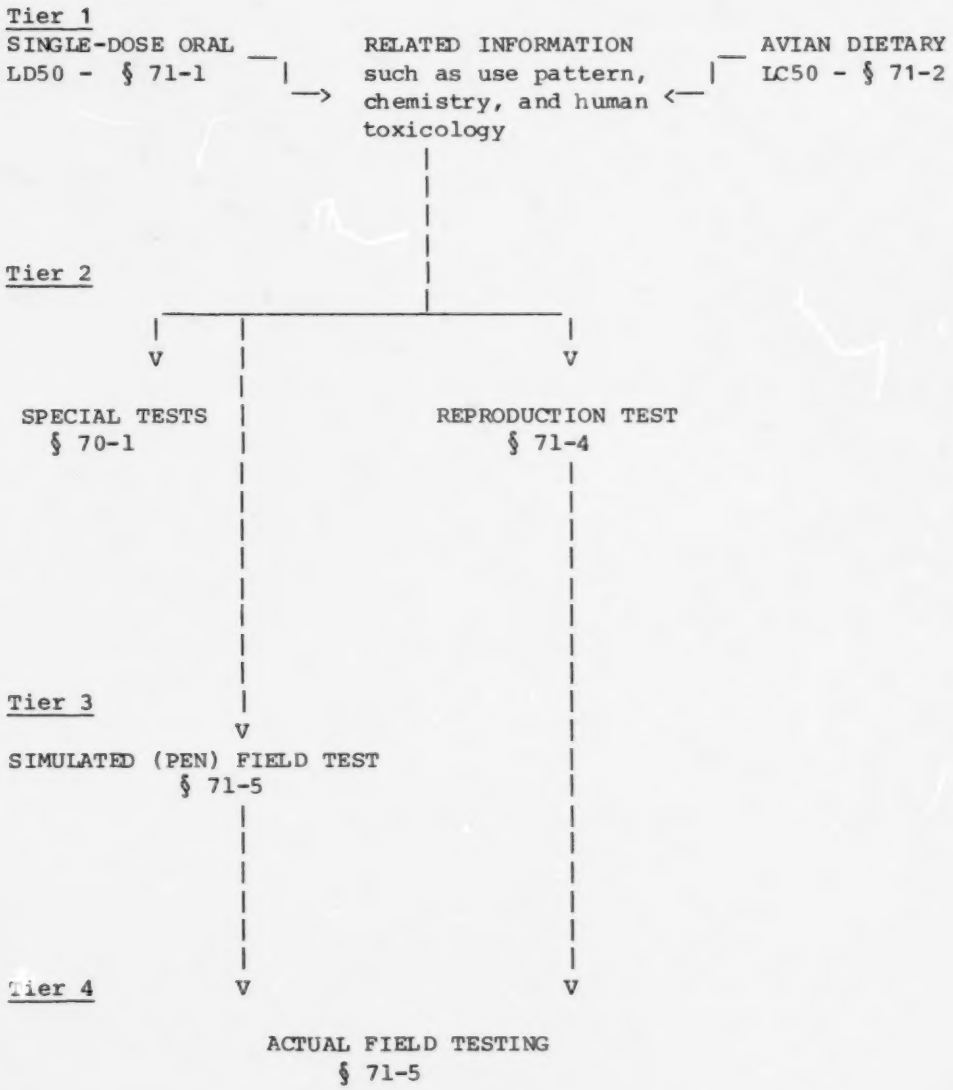

Tier 1
SINGLE-DOSE ORAL LD50 - § 71-1
RELATED INFORMATION such as use pattern, chemistry, and human toxicology
AVIAN DIETARY LC50 - § 71-2
Tier 2
SPECIAL TESTS § 70-1
REPRODUCTION TEST § 71-4
Tier 3
SIMULATED (PEN) FIELD TEST § 71-5
Tier 4
ACTUAL FIELD TESTING § 71-5

Figure 2. Wild Mammal Testing Flow Chart.

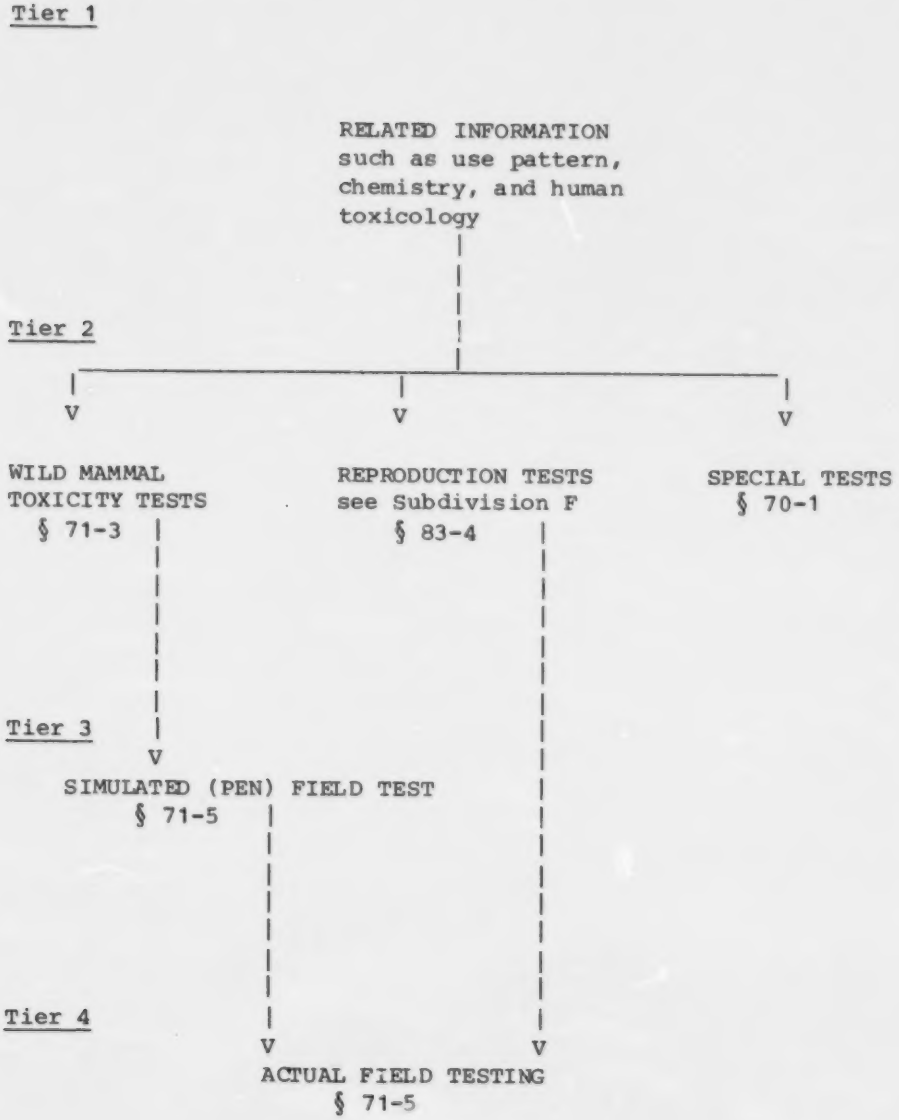
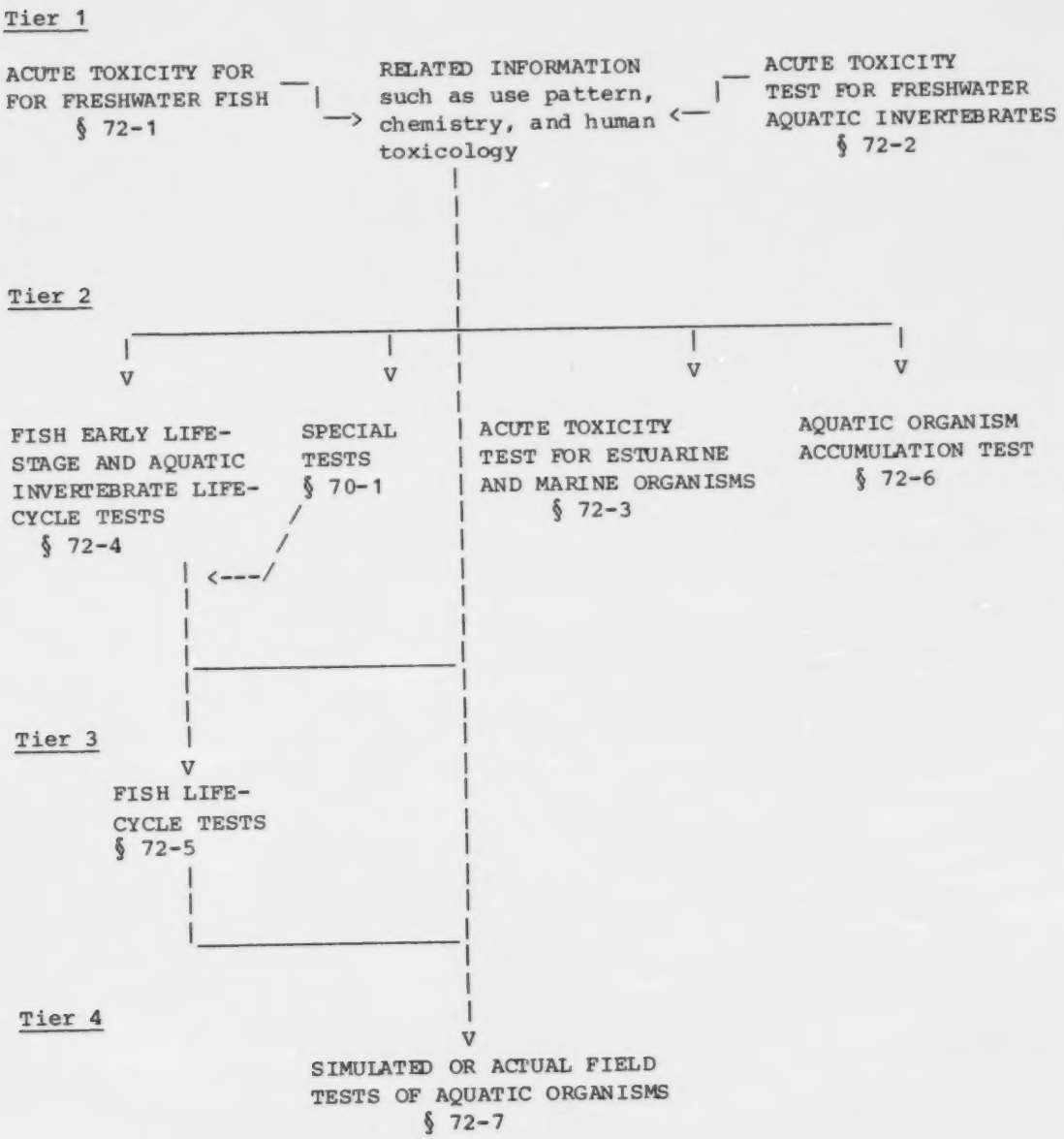


Figure 3. Aquatic Organism Testing Flow Chart.


Tier 1
ACUTE TOXICITY FOR FOR FRESHWATER FISH § 72-1
RELATED INFORMATION such as use pattern, chemistry, and human toxicology
ACUTE TOXICITY TEST FOR FRESHWATER AQUATIC INVERTEBRATES § 72-2
Tier 2
FISH EARLY LIFE-STAGE AND AQUATIC INVERTEBRATE LIFE-CYCLE TESTS § 72-4
SPECIAL TESTS § 70-1
ACUTE TOXICITY TEST FOR ESTUARINE AND MARINE ORGANISMS § 72-3
AQUATIC ORGANISM ACCUMULATION TEST § 72-6
Tier 3
FISH LIFE-CYCLE TESTS § 72-5
Tier 4
SIMULATED OR ACTUAL FIELD TESTS OF AQUATIC ORGANISMS § 72-7

(c) Range-finding tests. Unless the approximate toxicity of a test substance is already known, it is usually desirable to conduct a range-finding test to determine the concentrations of test substances that should be used in the definitive tests in this subdivision.

(d) Special test requirements. In addition to the testing guidance provided in all succeeding sections of this subdivision, data derived from additional tests may, under unusual circumstances, be required by 40 CFR § 158.75(a) the Agency for making judgments regarding safety to wildlife and aquatic organisms. Methods may be derived from tests already described or cited in this or other subdivisions of these guidelines. Such data requests may relate to a proposed pattern of use, a toxicological mode of action, or a unique chemical property. The data requested will be specific to the problem. Examples of tests for these unusual circumstances include but are not limited to:

(1) Avian acute dermal LD50;

(2) Certain toxicity data from Subdivision F, such as rabbit dermal LD50, eye irritation, and acute inhalation LC50;

(3) Fish or bird cholinesterase tests;

(4) Metabolism studies;

(5) Secondary toxicity studies on birds or mammals;

(6) Domestic animal safety studies (see Subdivision F, § 85-2);

and

(7) Pesticide efficacy testing, as outlined in Subdivision G, regarding pest control that may have major impact on the food supply or food chain of a rare or endangered species.

§ 70-2 Definitions.

(a) Terms used in this subdivision have the meanings set forth in 40 CFR § 162.3 and at § 60-2 of Subdivision D.

(b) In addition, for the purposes of this subdivision:

(1) The term "coldwater fish" means those fish which normally spawn in the fall and winter months in water colder than 60°F (15°C), e.g., fish from the family Salmonidae.

(2) The term "continuous exposure" means an extending or prolonged exposure usually resulting from a single application of a persistent pesticide.

(3) The term "EC50" (median effective concentration) is the concentration of a substance producing a specific effect or response in 50 percent of the test organisms. This determination is usually made when some effect other than lethality is being studied. The effect should be one which is easily observed and which is usually associated with lethality, e.g., failure of daphnids to demonstrate swimming response to light stimulus.

(4) The term "estimated environment concentration" (EEC) means an estimate of the concentration of a pesticide occurring in or on various media (i.e., soil, water, vegetation) after pesticide application, as determined from the results of environmental fate testing described in Subdivision N.

(5) The term "maximum expected environmental concentration" (MEEC) means the highest concentration of a pesticide that would occur [usually immediately after application(s)] at a site or in a medium (e.g., water, vegetation, or soil) contaminated with a pesticide, as determined from the pesticide application rate.

(6) The term "typical end-use product" means an end-use product representative of a major formulation category (e.g., emulsifiable concentrate, granular product, wettable powder) containing the active ingredient of the applicant's product.

(7) The term "warmwater fish" means those fish which normally spawn in the summer months in temperatures greater than 60°F (15°C), e.g., fish from the family Centrarchidae.

§ 70-3 General test standards.

(a) General policy. This section provides general test standards which apply to all tests in this subdivision. If, for a particular chemical, there should arise a conflict between the general standards and the standards for a particular test, the more specific standards should apply.

(b) Test methods. (1) Toxicity tests should be conducted according to uniform methods, whenever possible, to maximize the number of reliable comparisons that can be made concerning relative toxicity and relative sensitivity.

(2) Tests should include a concurrent control group. If a vehicle (carrier, solvent, or diluent) is used, the concurrent control group should be treated with that vehicle. Vehicles known to be toxic, synergistic, or antagonistic should not be used.

(3) All control groups should be maintained in the same manner as the test groups. However, control groups should be protected from airborne or other contamination by the test substance(s).

(4) In no case should the results of range-finding test(s) and definitive test(s) be combined in calculating the LD50, LC50, or EC50. Results of two or more definitive tests may be combined to calculate the LD50, LC50, or EC50 only if the tests are run concurrently using test animals which are from the same source and which were raised under the same conditions.

(c) Test substance. (1) Each section of Subdivision E provides guidance concerning the substance to be tested, i.e., whether the data submitted in support of an application for registration should be derived from tests conducted with the technical grade of the active ingredient, the end-use product, or a typical end-use product.

(2) The technical grade of the active ingredient is commonly the same substance as the manufacturing-use product for which registration is sought or which is used to produce the formulated pesticide product for which registration is sought. In this case, a sample of the manufacturing-use product may be tested in lieu of testing the technical grade of the active ingredient.

(3) In addition to or in lieu of testing otherwise provided by this subdivision, the Administrator may, in accordance with 40 CFR § 158.75(b), require testing to be conducted with:

(i) An analytically pure grade of an active ingredient;

(ii) The technical grade of an active ingredient;

(iii) The inert ingredient of a pesticide end-use formulation;

(iv) A contaminant or impurity of an active or inert ingredient;

(v) A plant or animal metabolite or degradation product of an active or inert ingredient;

(vi) The pesticide end-use formulation;

(vii) The pesticide end-use formulation plus any recommended vehicles and adjuvants;

(viii) Any additional substance which could act as a synergist to the product for which registration is sought; or

(ix) Any combination of the above substances.

(4) The test substance should be within the limits, if any, certified in accordance with the requirements of 40 CFR § 158.110.

(5) The test substance should be stored under conditions that maintain its stability.

(6) If a vehicle is used to dissolve or dilute the test substance, it should be chosen to possess as many of the following characteristics as possible:

(i) It should not interfere with absorption, distribution, metabolism, or retention of the test substance;

(ii) It should not materially alter the chemical properties of the test substance and not materially enhance, reduce, or alter the toxic characteristics of the test substance;

(iii) It should not materially affect the food and water consumption of the test organisms; and

(iv) At the levels used in the study, it should not produce physiological effects or have local or systemic toxicity. In addition, such a vehicle should, if possible, have a solvent polarity similar to the vehicle to be used in the end-use formulation.

(d) Test organisms. (1) All data submitted in support of an application for registration should be derived from tests conducted in accordance with good laboratory or field practices for handling and caring of test organisms. Only healthy organisms may be used, and they should be kept in conditions conforming to good husbandry or cultural practices. (See Animal Welfare Act, Pub. L. 94-279.)

(2) The organisms in each test should, as nearly as practicable, be of uniform weight, size, and age.

(3) Organisms should be randomly assigned to test groups to minimize bias and assure comparability of pertinent variables.

(4) The number of organisms tested per concentration, and the number of concentrations or dosage levels evaluated should be sufficient to yield statistically sound data.

(5) Organisms selected for testing should be species of wildlife and aquatic organisms currently established in the United States and its coastal waters, and should be phenotypically indistinguishable from wild species.

(6) In no circumstances shall threatened or endangered species be used as test organisms.

(7) Observations. Observations should be made as frequently as necessary to record all signs of intoxication and abnormal behavior.

§ 70-4 Reporting and evaluation of data.

(a) General policy. This section establishes general guidance for reporting and evaluation of data which apply to all tests in this subdivision. In the case of conflict between general and specific guidance for reporting and evaluation of data, the latter will govern.

(b) Basic standards for reporting and evaluation. (1) Each test report should include all information necessary to provide a complete and accurate description of test procedures and evaluation of the test results.

(2) Each test report should include a summary of the data, an analysis of the data, enough data for the Agency to verify calculated statistical values, and a statement of conclusions drawn from the analysis. The summary should be sufficient detail to permit the reader to independently understand the conclusions.

(3) The metric system should be used in test reports; the English system may also be used.

(c) Standards for specific aspects of reporting and evaluation. In addition to the guidance provided by other sections of this subdivision, each test report should include or reference the following information:

(1) Test method. (i) Statement of test method used and a full description of the experimental design and procedures [see also paragragh (c)(8) of this section];

(ii) The length of the study and the dates on which the study began and ended;

(iii) The name and address of the laboratory performing the test;

(iv) The location where the test was performed; and

(v) Name(s) of the principal investigator(s).

(2) Test substance. (i) Identification of the test substance, including composition, chemical name and percentage of active ingredient, molecular structure of the active ingredient, and qualitative and quantitative description of the chemical composition including the results of the analysis referenced in § 70-3(c)(4).

(ii) Manufacturer, lot and sample numbers of the test substance.

(3) Test organisms. (i) Identification of test organisms (scientific names);

(ii) Rationale for selection of species, if the species used is different than that preferred under the different sections of this subdivision.

(iii) Age, sex, size, life stage, and/or weights, as provided by the different sections of this subdivision;

(iv) Source of supply of the organisms;

(v) Method used in assigning test organisms to test and control groups;

(vi) Number of organisms per dose or concentration level and in control group;

(vii) Description of any pretest conditioning, including diet; and

(viii) History of colony, including record of observed diseases and disease treatments.

(4) Dosing or treatment. (i) Identification of method, route, and frequency of administration of test material, and randomization plan for treatments (when appropriate).

(ii) Length of treatment period;

(iii) Rationale for selection of method, route, or frequency, if different from that recommended in this subdivision;

(iv) Total volume of material administered (test substance plus carrier) to each test organism or in feed or water at each time administration is made;

(v) Any vehicle used to dissolve or dilute the test substance, or used to mix the test substance with the feed;

(vi) Doses or concentrations of test substances administered (i.e., milligrams of test substance per kilogram of body weight of the organisms or parts per million of the test substance in the feed or water) and method of determination of dose or concentration levels;

(vii) Dosing or treatment of control organisms; and

(viii) A description of the steps taken to maintain the concentration(s), if the test substance is administered in the feed or water and data from other subdivision(s) suggest that the actual concentrations over the length of the test may decrease by 30 percent or more.

(5) Observations. (i) Frequency, duration, and method of observation, including:

(ii) Detailed description of the nature, incidence, time of occurrence, severity, and duration of all observed toxic effects, including death and any other abnormal or unusual signs and symptoms; and

(iii) If gross pathological examinations are performed, a description of all abnormalties observed.

(6) Environmental conditions. (i) Wildlife species (mammals and birds). A description of the housing conditions during and, if known, prior to the test, including:

(A) Number of animals per cage (see Animal Welfare Act, Pub. L. 94-279);

(B) Ambient temperature and humidity;

(C) Photoperiod and lighting;

(D) A description of the diet, including identification and composition;

(E) Source of animal feed and water; and

(F) Dimensions of test pen(s).

(ii) Aquatic species (fish and invertebrates). A description of the test chamber conditions, including:

(A) Number of organisms per test chamber;

(B) The highest, lowest, and average water temperature;

(C) Photoperiod and lighting;

(D) A description of the diet, including identification and composition, and sources of feed;

(E) The source of dilution water, its chemical characteristics, and description of any pretreatment;

(F) Methods used for, and results of, all chemical analyses of water and all toxicant concentration determinations at beginning, during, and at end of tests, including validation studies and reagent blanks, if there is reason to suspect that the concentrations administered to the test water do not approximate the actual concentrations;

(G) The depth and volume of solution and dimensions of aquatic test chambers; and

(H) A description of the toxicant delivery system and flow rate, expressed as the average number of water volumes of test solution passing through each test chamber in 24 hours (recommended for flow-through aquatic tests).

(7) Data analysis. (i) Tabulation of the response data at each treatment level, including:

(ii) Calculation of the LD50, LC50, EC50, and the 95 percent confidence intervals when sufficient doses and test organisms are used to establish a dose-response line;

(iii) No-observed-effect level; and

(iv) Statistical method(s) used for analyzing data and a reference to it (them) in published literature. If there is a deviation from the published method, an explanation of the change and a rationale for its use should be included.

(8) References. (i) The Agency will accept references to the data as long as the complete text of the referenced data is readily available for Agency review.

(ii) The applicant should submit citations of protocols used for testing, or list references to any published literature and copies of unpublished literature used in developing the test protocol, performing the testing, making and interpreting observations, and compiling and evaluating the results.

Series 71: AVIAN AND MAMMALIAN TESTING

§ 71-1 Avian single-dose oral LD50 test.

(a) When required. (1) Data on the avian acute oral toxicity of a pesticide are required by 40 CFR § 158.145 to support the registration of an end-use product intended for outdoor application, and to support each application for registration of a manufacturing-use product which may be used to make such an end-use product.

(2) Data on the avian acute oral toxicity of a pesticide are required by 40 CFR § 158.145, on a case-by-case basis to support the registration of each end-use product intended solely for indoor application, and to support each application for registration of a manufacturing-use product which may be used to make such an end-use product.

(3) See 40 CFR § 158.50, "Formulators' Exemption," to determine whether these data must be submitted. Section II-A of this Subdivision provides an additional discussion on this subject.

(b) Test standards. Data sufficient to satisfy the requirements in 40 CFR § 158.145 should be derived from tests which comply with the general test standards in § 70-3 and the following test standards:

(1) Test substance. Data shall be derived from testing conducted with the technical grade of each active ingredient in the product.

(2) Species. Testing should be performed on one avian species, either wild waterfowl (preferably the mallard) or on an upland game bird (preferably the bobwhite quail). The species should be the same as one of the two species selected for the avian dietary LC50 test (§ 71-2).

(3) Age. Birds used in this test should be at least 16 weeks old at the start of testing. Within a given test, all birds should be from the same hatch.

(4) Determination of LD50. Satisfactory data should establish that the avian single-dose oral LD50 is greater than 2000 mg/kg, or establish an LD50 value and corresponding 95 percent confidence intervals.

(c) Reporting and evaluation of data. In addition to the information provided in § 70-4, the test report should contain:

(1) Age and sex of the birds tested;

(2) Mean body weights for each test and control group at initiation and termination of test;

(3) Diet used;

(4) Description of any antibiotics, vitamins, or food additives added to the feed preceding or during testing;

(5) Pen dimensions;

(6) Whether the pen is indoors or outdoors;

(7) Weather conditions, if pen is outdoors;

(8) Total feed consumption for each test and control group;

(9) Preparation of test material;

(10) Amount of test material dosed per bird;

(11) Amount of diluent dosed per bird, if used;

(12) Number of birds per treatment level;

(13) Number of controls used;

(14) LD50 in mg/kg, with 95 percent confidence limits;

(15) Method used for calculation of LD50;

(16) Slope of the dose response line;

(17) Time and date of mortalities;

(18) All signs of intoxication and regurgitation if any occurs; and

(19) Any gross pathological changes as noted by gross necropsies, if conducted.

(d) Acceptable protocol. The following is an example of an acceptable protocol for conducting an avian single-dose oral LD50 study. This study is a modification of a study that appears in an unpublished draft report to EPA from the American Institute of Biological Sciences (AIBS), titled Analysis of Specialized Pesticide Problems, Volume VI, Wildlife Toxicology Study, Pages 10 to 16. This report is dated October, 1974 and was funded under EPA Contract No. 68-01-2457.

Species and history. The test species of choice are pen-reared bobwhite and mallards not less than 16 weeks of age at initiation of test. Birds should not have been mated before being placed on test. A history of rearing practices such as photoperiod, medication, and type of food should be included in the report. All lots of birds should be healthy and of uniform size, weight, and parity. All lots should be weighed, then maintained for a minimum of 15 days prior to the test. If tested indoors, temperature and humidity should be controlled, with freedom from drafts and sudden noises which could disturb the test birds. Any lots of birds that suffer an abnormal weight loss or suffer more than 10 percent mortality during the holding period should not be used.

Cages. All birds should be caged by treatment level groups or subgroups under acceptable animal husbandry practices.

Weight. All birds should be weighed at the beginning of the test, and on day 14 after administration of the test substance. Also, birds should be weighed at the termination of the test if it extends beyond 14 days.

Diet and fasting. Feed should be withheld from the control and test birds (bobwhite and mallard) for 15 hours prior to oral administration of the chemical. At all other times, birds should have free access to feed. The diet should be standard feed ration known to be adequate for game birds. Feed consumption should be determined for treated and control birds. The feed consumed should be calculated as average daily food consumption for each dose level.

Preparation of test material. 1. The test material should be administered without the use of a vehicle, if possible. The test material should be accurately weighed and put directly in gelatin capsules. If the chemical is first dissolved in an evaporative vehicle (e.g., acetone, methylene chloride) in preparation for use with capsules, the evaporative vehicle should be completely evaporated at room temperature before the capsules are used.

2. If a vehicle is required, the preferred vehicle is distilled water. Other acceptable vehicles include table grade corn oil, propylene

glycol, 1 percent carboxymethylcellulose, and gum arabic (acacia). The recommended maximum amount of vehicle per dosage is 0.1 to 1 percent of body weight. The total volume for all dosage levels should approximate a constant volume-to-body weight factor.

3. A concurrent vehicle control is required. Vehicles known to be toxic, synergistic, or antagonistic should not be used. The vehicle should be administered to the control birds at the same volume as the administered to the test animals receiving the vehicle and the pesticide.

Methods of administration. The test chemical should be placed by oral intubation into the crop or proventriculus.

Number of birds per dose level. The number of test birds per treatment level and the number of controls should be no less than ten.

Dosage-mortality data. The factor between dosage levels used to calculate the acute LD50 should be based on a geometric or logarithmic scale. After preliminary screening, there should be a minimum of 5 dosage levels used in calculating the acute oral LD50. The calculation of an acute oral LD50 should follow any generally acceptable method. Two examples of acceptable methods are Thompson and Weil (1952) and Litchfield and Wilcoxon (1949).

In cases where the LD50 is found to be in excess of 2000 mg/kg, based upon the 10 or more birds per dosage level, data should show that less than half of the birds died at the 2000 mg/kg dosage level. If any birds die at this level, sequentially lower levels should be tested to provide a dose-response series which includes at least one level at which no mortality occurs.

Period of observation. The test animals should be observed for a minimum of 14 days. The observation period should be longer if signs of toxicity are still evident. The time of all deaths should be recorded by day. If more than 1 control bird (i.e., 10 percent) dies during the test period, the test may be considered invalid, depending on the circumstances and apparent cause of mortality.

Gross necropsy. Gross necropsies can give valuable additional data and they are encouraged, but are not required. When gross necropsies are conducted, they should be conducted on all dead birds and sufficient numbers of surviving birds to characterize any gross lesions in dead birds. Gross necropsies include general inspection of digestive tract, liver, kidneys, heart, and spleen. Any gross pathological changes shall be reported.

Recording of signs of intoxication. All signs of intoxication should be recorded as they occur.

(e) References. The following references can provide useful background information in developing acceptable protocols; some outline useful statistical procedures for handling data.

(1) Litchfield, L.J., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Therap. 96(2):99-133.

(2) Schafer, E.W., R.B. Bruntox, N.F. Lockyer, and J.W. Degrazio. 1973. Comparative toxicity of seventeen pesticides to the quelea, house sparrow and red-winged blackbird. Toxicol. Appl. Pharmacol. 26:154-157 (See also page 279.)

(3) Thompson, W.R., and C.S. Weil. 1952. On the construction of tables for moving average interpolation. Biometrics 8:51-54.

(4) Tucker, R.K., and D.G. Crabtree. 1970. Handbook on Toxicity of Pesticides to Wildlife. Fish and Wildlife Service Resource Publication 84. U.S. Dept. Interior, Washington, D.C. (See esp. pp 6 & 7.)

(5) Tucker, R.K., and M.A. Haegele. 1971. Comparative acute oral toxicity of pesticides to six species of birds. Toxicol. Appl. Pharmacol. 20:57-65. (See esp. pp. 57-59.)

§ 71-2 Avian dietary LC50 test.

(a) When required. (1) Data on the avian dietary toxicity of a pesticide are required by 40 CFR § 158.145 to support the registration of an end-use product intended for outdoor application, and to support each application for registration of a manufacturing-use product which may be used to make such an end-use product.

(2) Data on the avian dietary toxicity of a pesticide are required by 40 CFR § 158.145 on a case-by-case basis, to support the

registration of an end-use product intended solely for indoor application, and to support each application for registration of a manufacturing-use product which may be used to make such an end-use product.

(3) See 40 CFR § 158.50, "Formulators' exemption," to determine whether these data must be submitted. Section II-A of this Subdivision provides an additional discussion on this subject.

(b) Test standards. Data sufficient to satisfy the requirements 40 CFR § 158.145 should be derived from tests which comply with the general test standards in § 70-3 and the following test standards:

(1) Test substance. Data shall be derived from testing conducted with the technical grade of each active ingredient in the product.

(2) Species. Testing should be performed on two avian species: one species of wild waterfowl (preferably the mallard) and one species of upland game bird (preferably the bobwhite). One of the two species selected for these studies should be the same species selected for the avian acute oral LD50 study, § 71-1.

(3) Age. Bobwhite quail used in this study should be from 10 to 14 days old and mallards should be 5 to 10 days old at the beginning of the testing period. Within a given test, all birds should be from the same hatch.

(4) Determination of LC50. Satisfactory data should establish that the 8-day dietary (5 days treated diet and 3 days clean diet) LC50 is greater than 5000 ppm or establish an LC50 value and corresponding 95 percent confidence intervals.

(c) Reporting and evalution of data. In addition to the information provided in § 70-4, the test report should contain the following information:

(1) Age of birds (days);

(2) Breeding history of test birds;

(3) Source and strain of test birds;

(4) Mean body weights for each test and control group at initiation and termination of test;

(5) Test diets;

(6) Description of any antibiotics, vitamins, or food additives added to the feed preceding or during testing;

(7) Number of birds per concentration;

(8) Signs of intoxication (including unusual responses to chemical) by test birds;

(9) Total food consumption for each test and control group;

(10) LC50 determinations (ppm) with 95 percent confidence limits;

(11) Specific method used to calculate LC50's; and

(12) Slope of concentration response line.

(d) Acceptable protocols. The following are examples of acceptable protocols for conducting an avian dietary LC50 study.

(1) This protocol is a modification of a protocol that appears in an unpublished draft report to EPA from the American Institute of Biological Sciences (AIBS), titled Analysis of Specialized Pesticide Problems, Volume VI, Wildlife Toxicology Study, pages 17 to 22. This report is dated October, 1974 and was funded under EPA Contract No. 68-01-2457.

Test. This method determines a chemical's toxicity to birds expressed as the median lethal concentration (LC50) of the chemical (technical material) in dry diet [the LC50 being the parts per million (ppm) toxicant in ad libitum diet expected to produce 50 percent mortality among birds in 8 days (5 days on treated diet followed by at least 3 days on untreated diet)].

Species. This protocol is appropriate, with modification (e.g., acclimation to test diet), for most bird species, but it addresses primarily the mallard and bobwhite.

Source of strain of birds. Test birds should be pen-reared and phenotypically indistinguishable from wild birds. It is recommended that only birds be used from colonies that have had breeding histories maintained for them. It is also preferred that test birds be from the same hatch.

Age and sex. Bobwhite quail should be from 10 to 14 days old and mallard should be 5 to 10 days old at the initiation of the test. It should be noted that LC50 determinations may be affected by the age of the birds at the time of testing. Therefore, within a

given test all birds should be the same age. A random selection of birds without regard to sex may be used.

Health of birds. Only birds in apparent good health should be used. Birds with obvious abnormalities, injuries, or sickly appearance shall be excluded. Test birds should be preconditioned to the test facilities and untreated test diet for as long as possible prior to the beginning to the test.

Pen facilities. Pen conditions should conform to good husbandry practices. The facilities as described in the following paragraphs are considered to be satisfactory.

(1) Size. Bobwhite (about 10 birds per pen) may be tested in commercial brooder units with individual pens measuring approximately 35 x 100 x 24 cm high. Waterfowl (mallards) may be tested in pens 70 x 100 x 24 cm high. Floors and external walls can be of wire mesh, while ceilings and common walls may be of galvanized sheeting.

(2) Temperature and relative humidity. For bobwhites and mallards, brooder temperature should be about 35°C. It can be maintained with thermostatically controlled central heating. Temperature outside the brooder may range from 22-27°C. The relative humidity should generally be not less than 30 nor more than 80 percent. Adequate ventilation should be maintained.

(3) Lighting. Fluorescent or incandescent lighting may be supplied with varying light regimes. A diurnal photoperiod is recommended; however, lighting maintained continuously, i.e. 24 hours per day, is also acceptable.

(4) Pen locations. Generally, studies conducted indoors provide better control of environmental conditions, and, if predators are a problem, better protection from predation. However, studies can be conducted outdoors and still provide suitable data.

(5) Test diets. A standard commercial game bird diet (mash form) or its equivalent should be used. Water should be continuously available. The test material should be added to the diet without the use of a vehicle, if possible. If a vehicle is used, the preferred vehicle is distilled water. However,

when a test chemical is not water-soluble, the test material may be dissolved in a reagent grade evaporative vehicle (e.g., acetone, methylene chloride) and mixed with the test diet. The vehicle must be completely evaporated at room temperature prior to feeding. Other acceptable vehicles include table grade corn oil, propylene glycol, 1 percent carboxymethylcellulose, and gum arabic (acacia). The vehicle and the dissolved test material should be mixed thoroughly with dry commerical mash in a ratio of 2 parts of solution to 98 parts of feed by weight. An equivalent amount of vehicle should be added to untreated diets. Diets can be mixed by commercial mechanical food mixers. Mashes and toxicant should be freshly mixed in small quantities. Then mixtures should be combined with larger quantities of mash to provide uniformity in the final dietary concentration of pesticide in the mash.

Concentrations and dosage mortality data. Generally, each chemical should be administered in at least 4 dietary concentrations (but 5 or 6 are preferred) spaced geometrically over a span intended to produce mortality ranging from 10 to 90 percent. It is advisable to perform a range-finding study prior to selecting concentrations for testing.

Ten birds per concentration and 10 birds for the control group are recommended. Experimental variation may require more than 10 birds, and in some cases fewer than 10 birds may be used.

In cases where the LC50 is found to be in excess of 5000 ppm by dietary exposure, based upon the 10 or more birds per dosage level, data should show that less than half the birds died at the 5000 ppm dosage level. If any birds die at this level, sequentially lower levels should be tested to provide a dose-response series which includes at least one level in which no mortality occurs.

Observations on signs of intoxication. Throughout the test, all signs of intoxication and abnormal behavior should be noted. Any unusual manifestation of the chemical to the test birds should be recorded. The period after the 5-day exposure of birds to treated diets should be extended for at least 3 days or for as long as test birds exhibit toxic signs and continue to die.

Food consumption. Estimates of average food consumption should be made for each pen of birds in each test. Care should be taken to see that feed spillage or air contamination by volatile chemicals from pen to pen does not take place.

Statistical procedure for handling data. The LC50 values and associated statistics can be derived by methods of probit analysis as described by Finney (1971) and programmed for computer by Daum and Killcreas (1966). The application of the basic probit method by Litchfield and Wilcoxon (1949), Miller and Tainter (1944), or other appropriate sources may be utilized.

(2) ASTM standard E-857-81, Standard practice for conducting subacute dietary toxicity tests with avian species. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

(e) References. The following references can provide useful background information in developing acceptable protocols; some outline useful statistical procedures for handling data.

(1) Daum, R.J. 1970. Revision of two computer programs for probit analysis. Bull. Entomol. Soc. Am. 16(1):10-15.

(2) Daum, R.J., and W. Killcreas. 1966. Two computer programs for probit analysis. Bull. Entomol. Soc. Am. 12(4):365-369.

(3) Finney, D.J. 1971. Probit Analysis. 3rd Ed. Cambridge University Press: London and New York.

(4) Heath, R.G., J.W. Spann, J.R. Kreitzer, and C. Vance. 1970. Effects of polychlorinated biphenyls on birds. Presented at XV International Ornithological Congress, The Hague, 30 Aug. - 5 Sept., 1970. Pp. 475-485 in Proceedings of the XV Int. Ornith. Congress. K.H. Voous, ed. Published by E.J. Rill, Leiden.

(5) Heath, R.G., and J.W. Spann. 1973. Reproduction and related residues in birds fed mirex. Pp. 421-435 in Pesticides and the Environment: A Continuing Controversy. Wm. B. Deichman, ed. InterContinental Medical Book Corp. N.Y., N.Y. (Selected Papers, 8th Inter-Amer. Cong. on Toxicol. & Occupat. Med., Univ. of Miami, School of Med., Miami, Florida.)

(6) Hill, E.F., R.G. Heath, J.W. Span, and J.D. Williams. 1975. Lethal dietary toxicities of environmental pollutants to birds. U.S. Fish and Wildlife Service Special Scientific Report - Wildlife No. 191. U.S. Dept. Interior, Wash., D.C. 61 pp.

(7) Litchfield, J.T., Jr., and F. Wilcoxin. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Therap. 96(2):99-133.

(8) Miller, L.C., and M.L. Tainter. 1944. Estimation of ED50 and its error by means of logarithmic-probit graph paper. Proc. Soc. Exp. Biol. 57:261-264.

(9) Weil, C.S. 1952. Tables for convenient calculation of median-effective dose (LD50 or ED50) and instructions in their use. Biometrics 8:249-262.

§ 71-3 Wild mammal toxicity test.

(a) When required. (1) Data on the toxicity of a pesticide to wild mammals are required by 40 CFR § 158.145 on a case-by-case basis to support the registration of an end-use product intended for outdoor application. The toxicity data required by 40 CFR § 158.135 for evaluating hazards to humans and domestic animals are normally adequate to indicate hazard to wild mammals. Under certain conditions, however, these data are not sufficient to assess the potential hazard to wild mammals which are likely to be exposed to an end-use product. Examples of circumstances when data on the toxicity of a pesticide to wild mammals may be required by 40 CFR § 158.145 include, but are not limited to, the following:

(i) When data (e.g., data required by 40 CFR § 158.135) indicate that there is considerable variation in the sensitivity of different mammalian species to the toxic effects of a pesticide, and when there is evidence that wild mammals are heavily exposed to pesticides; and

(ii) When pesticides with bactericidal properties will be applied to the forage of wild ruminants, and toxicological data do not include information on possible interference with rumen fermentation in domestic (or wild) ruminants.

(2) See 40 CFR § 158.50, "Formulators' exemption," to determine whether these data must be submitted. Section II-A of this Subdivision provides an additional discussion on this subject.

(b) Test standards. Data sufficient to satisfy the requirements in 40 CFR § 158.145 should be derived from tests which comply with the general test standards in § 70-3 and all of the following standards:

(1) Test substance. (i) Generally, data shall be derived from testing conducted with the technical grade of each active ingredient in the product.

(ii) In special circumstances, data from testing conducted with the applicant's end-use formulation or a typical end-use product may be required by 40 CFR § 158.75(b) to support the registration of an end-use product. Examples of such circumstances include, but are not limited to:

(A) When effects of the product on the rumen fermentation process need to be determined, or

(B) When secondary toxicity studies need to be performed (e.g., product is introduced into prey species which is fed to predator species).

(2) Species. Testing should be performed on a mammalian species representative of those found in the area(s) likely to be affected by the proposed use pattern(s). Test animals may be pen-reared or wild captured, but should be phenotypically indistinguishable from wild mammals. In no case should endangered or threatened animals to be used for testing.

(3) Toxicity determination. When the animals are large or the species is relatively scarce, a study which determines only the approximate maximum tolerated dosage for the test species may be acceptable. In all other cases, the acute oral LD50, dietary LC50, or dietary no-effect level should be determined, following consultation between the Agency and the registrant.

(c) Reporting and evaluation of data. In addition to the information provided in § 70-4, test reports should contain the following information:

(1) Age of each animal and how determined;

(2) Mean body weight for each test and control group at initiation and termination of test;

(3) Number of animals of each sex of animal tested;

(4) Total food consumption for each test and contrcl group;

(5) Test diet;

(6) Dose schedule;

(7) Mortality;

(8) Number and circumstances of accidental deaths or injuries;

(9) LD50 (in mg/kg) or LC50 (in ppm) with 95 percent confidence limits, or estimated maximum tolerated dose;

(10) Specified methods used to calculate LD50 or LC50; and

(11) Slope of the dose-response line.

(d) Acceptable protocols. Because the Agency intends that toxicity tests on mammals, other than those required by 40 CFR § 158.135, be conducted only to access specific situations, no single test methodology can provide adequate guidance for all cases. In addition, there are no widely accepted protocols that include husbandry practices appropriate to a diversity of mammals. Therefore, the test methodologies should be determined on a case-by-case basis. Appropriate tests methods should be developed by the registrant in consultation with the Agency. The following are offered for guidance.

(1) Dietary LC50 and no-effect level tests. Methods for dietary tests may be adapted from the subchronic oral dosing studies for mammals in Subdivision F, § 82-1, and/or from the avian dietary LC50 study in this Subdivision at § 71-2. See § 71-3(e) for references.

(2) Toxicity studies for large and relatively scarce mammals. An example of an acceptable protocol for toxicity studies with mammals that are large, relatively scarce, or otherwise difficult to obtain is provided below. This protocol is a modification of a protocol that appears in an unpublished draft report to EPA from the American Institute of Biological Sciences (AIBS), titled Analysis of Specialized Pesticide Problems, Volume VI, Wildlife Toxicology Study, pages 4 to 9. This report is dated October, 1974 and was funded under EPA Contract No. 68-01-2457.

Introduction

The purpose of this test is to compare the toxic effect(s) of a pesticide on different species of wild mammals. As such, precise LC50 values are not always essential; rather, the approximate maximum tolerated dose can be estimated and the character of the toxic syndrome determined. Careful observation and thorough necropsy examination are required. A preliminary range-finding test should be first conducted to establish the approximate lethal dose, and subsequent tests with dosing of additional animals (in amounts determined according to the geometric progression indicated by the results of standard mammalian acute toxicity) should be conducted to establish the approximate maximum tolerated dose. A single and/or multiple dosing schedule may be used for the test. The latter schedule, if extended for 10 days or more, can be used to indicate the relative chronicity of the pesticide in the wild species in place of an extended subacute toxicity schedule. After dosing, animals should be observed for several days for delayed toxic effects. Beyond simple mortality, signs of intoxication such as loss of weight, anorexia, and diarrhea are important. The general procedures followed in testing the toxicity of herbicides in cattle and sheep by the U.S. Department of Agriculture's Veterinary Toxicology and Entomology Research Laboratory (College Station, Texas), Science and Education Administration (formerly Toxicological Investigation laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service) are typical and representative of common practice.

Methods and Materials

The best policy to follow, within the constraints dictated by the particular species selected, is to exercise the following sound principles of animal selection and management. Animals should be in healthy conditions, as free of social and environmental stress as possible, acclimated to diet, and uninfluenced by drugs. Normally, adults of similar age, judged at least by body weight (but preferably by additional methods as well), should be used, and data from both sexes should be obtained if possible. Use of reared stock is generally preferable to live-trapped animals.

Animal conditioning. Animals selected from appropriate sources should be allowed a prolonged period of acclimatization to the test facilities and feed. Use of replicate controls is desirable in tests with wild mammals.

Dose selection. Dosage should be calculated on a mg/kg body weight basis for the active component(s) of each chemical tested. Selection of the dosage rates for each type of test animal involves several variables. The initial dosage level should be estimated from existing acute toxicity data, and the toxic range found by trial and error. When a toxic dosage is found, additional dosages above and below this rate should then be applied to the other animals. Where a step-by-step increase of dosage indicates increased toxicity, repetition of individual dosage is not necessary.

Chemical administration. Oral dosing should be accomplished by placement in the stomach of gelatin capsules containing the test substance. A balling gun, or "drenching" (in which a solution or suspension of the chemical is introduced into the stomach by syringe and stomach tube), may be used. Occasionally it is necessary to conceal the dosing in an attractive food item. Drenching usually involves water-diluted preparations as compared to undiluted material placed in capsules. Data from the U.S. Department of Agriculture's Veterinary Toxicology and Entomology Research Laboratory indicate general inconsistency between these forms of dosing, but no generalization is possible favoring one over the other. Use of a vehicle such as corn oil and its volume may also affect the acute toxicity evaluation. For the immediate purpose of interspecies comparisons, the solvent or dispersant should match that used in the acute oral toxicity testing (i.e., § 81-1 of Subdivision F guidelines). The volume administered orally should never exceed 3 percent of the body weight and should be constant at all dosage levels.

Absorption or effects related to the amound of feed in the digestive tract are minimized by overnight starvation prior to administration of the chemical dose. Feed should be provided following dosing, and water ad libitum prior to and following dosing. Withholding feed from ruminants serves little purpose; it is preferable to maintain such animals on restricted feed allotments calculated to maintain body weight.

Dosing schedule and test design. Testing may use a single and/or a multiple dose schedule. If the single dose schedule is used, the test animals should be observed for approximately ten days following the dose for signs of toxic effect, such as loss of weight, anorexia, and abnormal function or behavior. If a multiple dose schedule is used, the test animal should be observed during a series of 10 or more daily administrations and a minimum of 10 days following the last administered dose.

(e) References. The following references can provide useful background information in developing acceptable protocols:

(1) Large and relatively scarce mammals.

Agr. Res. Service, U.S.D.A. Animal Disease and Parasite Research Division. 1969. The toxicity of some organic herbicides to cattle, sheep, and chickens. A.R.S. Production Research Report No. 106. U.S. Dept. Agriculture, Wash., D.C.

(2) Small mammals LC50. The following reference contains an acceptable protocol for determining the dietary toxicity in small animals.

McCann, J.A., Teeters, W., Urban, D.J., and Cook, N. 1981. "A Short Term Dietary Toxicity Test on Small Mammals," Avian and Mammalian Wildlife Toxicology: Second Conference, ASTM STP 757, D.W. Lamb and E.E. Kenaga, Eds., American Society for Testing and Materials, Pp. 132-142.

§ 71-4 Avian reproduction test.

(a) When required. (1) Data on avian reproductive effects are required by 40 CFR § 158.145 to support the registration of an end-use product which meets one or more of the following criteria:

(i) Its labeling contains directions for using the product under conditions where birds may be subject to repeated or continuous exposure to the pesticide or any of its major metabolites or degradation products, especially preceding or during the breeding season.

(ii) The pesticide or any of its major metabolites or degradation products are stable in the environment to the extent that potentially toxic amounts may persist in avian feed.

(iii) The pesticide or any of its major metabolites or degradation products is stored or accumulated in plant or animal tissues, as indicated by the partition coefficient of lipophilic pesticides (§§ 165-3, -4, and -5 of Subdivision N) metabolic release and retention studies (§ 85-1 of Subdivision F), or as indicated by structural similarity to known bioaccumulative chemicals.

(iv) Any other information, such as that derived from mammalian reproduction studies (§ 83-4 of Subdivision F), that indicates the reproduction in terrestrial vertebrates may be adversely affected by the anticipated use of the pesticide product.

(2) Applicants for registration of avicides should consult with the Agency prior to conducting this test.

(3) See 40 CFR § 158.50, "Formulators' exemption," to determine whether these data must be submitted. Section II-A of this Subdivision provides an additional discussion on this subject.

(b) Test standards. Data sufficient to satisfy the requirements in 40 CFR § 158.145 should be derived from tests which comply with the general test standards in § 70-3 and all of the following test standards:

(1) Test substance. Data shall be derived from testing conducted with the technical grade of each active ingredient in the product.

(2) Species. Testing should be performed on the bobwhite quail and mallard.

(3) Dose levels. At least two treatment level groups and a vehicle control group should be used.

(4) Number of test animals. When other test data reveal bioaccumulative potential, the number of test animals in the test group should be increased sufficiently to partly offset animal deaths or data-gathering problems associated with morbidity or with tissue residue determinations.

(5) Age. Birds approaching their first breeding season should be used.

(6) Duration of administration. Birds should be exposed to treated diets beginning not less than 10 weeks before egg laying is expected, and extending throughout the laying season.

(c) Reporting and evaluation of data. In addition to the information provided in § 70-4, the test report should contain:

(1) Test results. The following information, reported for all test groups:

(i) All observed abnormal behavior;

(ii) All observed morphological and physiological responses;

(iii) Post mortem autopsy.

(2) Test conditions. The following information, reported for each treated and untreated test group:

(i) Species;

(ii) Strain;

(iii) Age;

(iv) Body weight;

(v) Number of birds per test (include sex ratio);

(vi) Individual identification of birds;

(vii) Diet;

(viii) Storage;

(ix) Feed consumption (grams per day);

(x) Observation on palatability or repellency;

(xi) Housing conditions of test birds, including:

(A) Space allocations for mating and nesting;

(B) Protection from weather and injuries; and

(C) Lighting program, including hours per day and wattage or foot candles at bird level;

(xii) Diagram of test layout;

(xiii) Temperature;

(xiv) Water supply;

(xv) Pretest and test history or medical and chemical administration; and

(xvi) Length of treatment period and observation period.

(3) Egg and hatching data. The following information, reported for each treated and untreated test group:

(i) Egg shell thickness;

(ii) Number and percent of cracked eggs;

(iii) Eggs laid (number eggs per bird per day and per season);

(iv) Hatching egg storage data:

(A) Temperature;

(B) Humidity;

(C) Incubation data;

(D) Eggs set; and

(E) Egg turning frequency;

(V) Fertility (viable embryos);

(vi) Live 3-week embryos;

(vii) Embryos that mature, embryos that pip shell, and embryos that liberate themselves, and a determination of hatchability;

(viii) Dead embryos;

(ix) Fourteen-day-old survivors;

(x) Crippled survivors;

(xi) Post-hatching mortability;

(xii) Weights of fourteen-day-old survivors; and

(xiii) Any signs of intoxication in post-hatching survivors.

(4) Feed analysis data. Levels of concentration of pesticide in the feed used in each test, and the rationale for choice of such levels.

(d) Acceptable protocol. Except where noted, the following example of avian reproduction protocol is acceptable for the testing of both bobwhites and mallards. This study is a modification of a study that appears on pages 23 to 50 in an unpublished draft report to EPA from the American Institute of Biological Sciences (AIBS), titled analysis of Specialized Pesticide Programs, Volume VI, Wildlife Toxicology Study. The report is dated October, 1974, and was funded under EPA Contract No. 68-01-2457.

Test animals. Pen-reared birds, previously untreated, approaching their first breeding season, and phenotypically indistinguishable from wild birds, should be used as test animals. If shipped, all birds should be examined following shipment for possible physical injury that may have been encountered in transit. If deemed necessary, several birds may be randomly selected for pretreatment necropsy at a diagnostic laboratory to assess the state of health upon arrival. It is desirable to have a 2- to 6-week health observation period prior to selection of birds for treatment.

A history of rearing practice for the birds to be tested should be obtained if possible. This history should include lighting practices during rearing, disease record, drug and any other medication administered, and exact age.

Test groups - Bobwhite. A minimum of 3 test groups of bobwhite should be used. One group should serve as a control and 2 groups as treated birds. By random distribution, 1 male and 2 females per pen, replicated by a minimum of 12 pens, should be used per group. If individual pairs (1 male and 1 female) are to be used per pen, more pens (greater than 12) per test group should be used to proide similar sensitivity to the group testing design. To determine the number of pens needed for a particular level of sensitivity, see Walpole and Myers (1972). Control and treated birds should be kept under the same experimental conditions.

Test groups - Mallards. A minimum of 3 test groups of mallards should be used. One group should serve as a control and 2 groups as treated birdss. By random distribution, 2 males and 5 females per pen, replicated by 5 or more pens, should be used per group. If individual pairs (1 male and 1 female) are to be used per pen, considerably more pens (greater than 12 per test group should be used to provide similar sensitivity to the group testing design. To determine the number of pens needed for a particular level of sensitivity, see Walpole and Myers (1972). Control and treated birds should be kept under the same experimental conditions.

Diet preparation. Concentrations for the test substance should be based on measured or calculated residues expected in the diet from the proposed use pattern(s). The concentrations should include an actual or expected field residue exposure level and a multiple level such as five. The highest nonlethal level may be estimated from data developed from the avian dietary LC50 (§ 71-2).

The test material should be added to table grade corn oil or other appropriate vehicle and premixed with an aliquot of basal diet, utilizing a mortar and pestle or mechanical blender. It is recommended that the aliquot of basal diet used for the premix be screened to remove large particles of diet before blending in the corn oil and test material. The final diet should be a uniformly mixed composition consisting of 98 to 99 parts by weight of basal diet and 1 or 2

parts by weight of corn oil. The basal diet should be a commercial game bird breeder ration (or its equivalent) that is treated with an equivalent amount of vehicle. The premix should be stored under conditions which maintain stability. Test diets should be analyzed for pesticide concentrations at intervals during the tests. If other long-term animal tests have demonstrated a propensity for the test chemical to persist or bioaccumulate, the degree of bioaccumulation in birds should be determined by measurement of tissue residues in the birds from an extra pen group put through the reproduction test. Two or three tissues should be selected for residue analysis at the end of the exposure period, based on tissues known from other studies to hold highest residues.

Testing phase - test environment. The birds should be housed in breeding pens of adequate size conforming to good husbandry practices. The mallard pens should be screen-bottomed or kept clean of spilled food and excrement. It is desirable to offer mallards water in which to bathe.

Since light is extremely important, both during rearing and during the egg laying period, all birds should be maintained for the first 8 weeks under a regime of 7 hours of light per day for maximum egg production.

The photoperiod should then be increased to 16-17 hours of light per day and either maintained at this level or increased by 15 minutes per week for the following 12 weeks. (The 12-week period may vary depending upon the time required for the onset of egg production.) An illumination intensity of 6 footcandles at the bird level during the lighting phase of the reproductive study is adequate. Avoid the use of shorter wavelength "cool white" fluorescent lights which do not emit the daylight spectrum.

Temperature and relative humidity control throughout the reproductive test is desirable and should be recorded. Recommended levels are 21°C and 55 percent relative humidity. Ventilation is necessary.

Feeding and husbandry. All birds should receive the appropriate diet ad libitum for the duration of the study. Water is to be provided ad libitum. The test chemical should be administered for at least 10 weeks prior to the onset of egg laying.

Body weights should be recorded at test initiation prior to onset of laying, and at termination. During egg laying, body weight recording is discouraged because of the adverse effects that handling may have on egg production.

Food consumption should be recorded at least at biweekly intervals throughout the study.

Mortality should be recorded by date and morbidity (noted together with clinical signs) throughout the test phase. Gross pathology data should be obtained for birds that die during the course of the test phase and for some survivors.

Egg collection, storage, and incubation. All eggs should be collected daily, marked according to pen from which collected, and stored at 16°C and 65 percent relative humidity. Eggs should be set at weekly intervals for incubation in a commercial incubator. All eggs should be candled on day 0 for eggshell cracks; on approximately day 11 for bobwhites and day 14 for mallards to measure fertility and early death of embryos; and on day 18 for bobwhite and day 21 for mallards to measure embryo survival. For hatching, transfer of the eggs to a separate commercial incubator or hatcher should be made on day 21 for bobwhites and Day 23 for mallards.

Recommended temperatures and relative humidity during hatching phase are 39°C and 70 percent, respectively.

Bobwhite chick observations. On Day 24 of incubation, the hatched bobwhite chicks should be removed, hatchability recorded, chicks housed according to the appropriate parental grouping, and maintained on control diet for 14 days. The time period should be extended if mortality occurs appreciably late. The diet should be a commercial bobwhite starter diet or its equivalent.

Duckling observation. On Day 27 of incubation, the hatched mallard ducklings should be removed, hatchability recorded, ducklings housed according to the appropriate parental grouping, and maintained on control diet for 14 days. The time period should be extened if mortality occurs appreciably late. The diet should be commercial mallard starter diet or its equivalent.

Eggshell thickness. One day every two weeks newly laid eggs should be collected and measured for eggshell thickness. For consistency, the eggs used for thickness determinations should be collected during weeks 1, 3, 5, 7 and 9 of the egg-laying period. An accepted procedure is to crack open the eggs at the widest portion (girth or waist), wash out all egg contents, air-dry the shells for at least 48 hours, and then measure the thickness of the dried shell plus the membranes at 3 or 4 points around the girth using a micrometer calibrated to 0.01 mm units.

Analysis. Reproductive data consists of continuous variables (e.g., shell thickness, and body weight data) and discrete variables (e.g., number of eggs laid or 14-day-old survivors). For continuous variables, experimental groups should be compared to controls by analysis of variance. For most discrete variables, survival percentages should be computed (e.g., 14-day-old survivors of eggs laid) and arcsine transformed prior to analysis of variance. Alternately, a chi square analysis of survival (contingency tables) may be used for discrete variables. Analyses should include: body weight, food consumption, eggs laid, eggshell thickness, eggs cracked, viable eggs, fertility, live 3-week embryos, hatchability, number of normal chicks or ducklings, 14-day-old survivors (per number of eggs hatched, per hen, and per number of eggs laid). Sample units are generally the pens within each group.

Withdrawal. If the test substance is toxic (reduced reproduction evident), then a withdrawal study period should be added to the test phase. The withdrawal period need not exceed 3 weeks. Continued observations should be made on egg production, fertility, hatchability, and hatching survival.

Definitions:

1. Eggs laid. The total egg production during a breeding season (which is approximately 10 weeks).

2. Eggs cracked. Eggs determined to have cracked shells when inspected with a candling lamp; fine cracks cannot be detected without utilizing a candling lamp and if undetected will bias data by adversely affecting embryo development.

3. Eggs set. All eggs placed under insubation, i.e., total eggs laid minus cracked eggs and those selected for eggshell thickness analysis.

4. Viable embryos (fertility). Eggs in which fertilization has occurred and embryonic development has begun. This is determined by candling the eggs 6 to 14 days after incubation has begun. It is difficult to distinguish between the absence of fertilization and early embryonic death. This distinction can be made by breaking out eggs that appear infertile and examining further. This is especially important when a test compound induces early embryo mortality.

5. Live 3-week embryo. Embryo that is developing normally after 3 weeks of incubation. This is determined by candling the egg.

6. Hatchability. The percentage of embryos that mature, pip the shell, and liberate themselves from their eggs as computed from the number of fertile eggs. For quail this generally occurs on day 23 or 24 of incubation, and for mallard on day 25, 26, or 27.

7. 14-day-old-survivors. Birds that survive for 2 weeks following hatch.

8. Eggshell thickness. The thickness of the shell and the membrane of the egg at the girth after the egg has been opened and washed out, then the shell with membrane dried for at least 48 hours at room temperature.

(e) References. The following references can provide useful background information in developing acceptable protocols; some outline useful statistical procedures for handling data.

(1) Cochran, W.G. 1943. Analysis of variance for percentages based on unequal numbers. Am. Stat. Assoc. 38:287-301.

(2) Davidson, K.L., and J.L. Sell. 1974. DDT thins shells of eggs from mallard ducks maintained on ad libitum or controlled-feeding regimes. Arch. Environ. Contam. Toxicol. 2(3):222-232.

(3) Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11:1-42.

(4) Heath, R.G., J.W. Spann, and J.F. Kreitzer. 1969. Marked DDE impairment of mallard reproduction in controlled studies. Nature 224(5215):47-48.

(5) Heath, R.G., J.W. Spann, J.R. Kreitzer, and C. Vance. 1970. Effects of polychlorinated biphenyls on birds. Presented at the XV Internat. Ornith. Congress, The Hague, 30 Aug - 5 Sept., 1970. Pp. 475-485 in Proceedings of XV Internat. Ornith. Congress. K.H. Voous, ed. E.J. Brill, (pub.) Leiden.

(6) Heinz, G. 1974. Effects of dietary levels of methyl mercury on mallard reproduction. Bull. Env. Cont. Toxicol. 11:386-392.

(7) Longcore, J.R., F.B. Sampson, and T.W. Whittendale, Jr. 1971. DDE thins eggshells and lowers reproductive success of captive black ducks. Bull. Env. Cont. Toxicol. 6:485-490.

(8) Prince, H.H., P.B. Seigel, and G.W. Cornwell. 1969. Incubation environment and the development of mallard embryos. J. Wildlife Manage. 33:589-595.

(9) Stromborg, K.L., 1981. Reproductive test of diazinon on bobwhite quail, Avian and Mammalian Wildlife Toxicology: Second conference, ASTM STP 757, D.W. Lamb and E.E. Kenaga, Eds., American Society for Testing and Materials, pp. 19-30.

(10) Walpole, R.E. and R.H. Myers. 1972. Probability and statistics for engineers and scientists. The MacMillan Company, New York. Pp. 387-392.

§ 71-5 Simulated and actual field testing for mammals and birds.

(a) When required. (1) Data from any of the following kinds of tests are required by 40 CFR § 158.145, on a case-by-case basis, to support the registration of an end-use product intended for outdoor application. Consultation with the Agency is advised before undertaking these tests. Whenever data are required by 40 CFR § 158.145, the determination will be made in writing by the Agency and will state which properties and use patterns of the product were used in the determination. The following criteria are provided as further guidance:

(i) Simulated (pen) field tests are required by 40 CFR § 158.145 to support the registration of an end-use formulated product if use of the pesticide is likely to result in adverse effects on wildlife organisms exposed to the pesticide, and if pen field tests can yield data useful in assessing such risks. A decision as to whether such a test is needed should take into account:

A. An analysis of the pesticide properties (e.g., persistence, conversion to toxic metabolites);

B. Retention on food, and repellency;

C. Intended use patterns (e.g., treated habitats, expected presence of species, and treatment amounts at toxic levels after application); and

D The results of other laboratory tests.

(ii) Actual field tests are required by 40 CFR § 158.145 to support the registration of an end-use formulated product if use of the pesticide is likely to result in adverse effects on wildlife exposed to the pesticide, and if actual field tests can yield data useful in assessing such risks. A decision as to whether such a test is needed should take into account:

(A) An analysis of the pesticide properties (e.g., persistence, conversion to toxic metabolites, retention on food, and repellency);

(B) Intended use patterns (e.g., treated habitats, expected presence of species, and treatment amounts at toxic levels after application); and

(C) Evidence (e.g., reported field effects, simulated pen studies or chronic lab studies) demonstrating acute toxicity at normal use rates or demonstrating long-term chronic or reproductive effects.

(2) See 40 CFR § 158.50, "Formulators' exemption," to determine whether these data must be submitted. Section II-A of this Subdivision provides an additional discussion on this subject.

(b) Test standards. Data sufficient to satisfy the requirements in 40 CFR § 158.145 should be derived from tests which satisfy the purposes of the general test standards in § 70-3 and the following test standards:

(1) Test substance. Unless specified otherwise, data shall be derived from testing conducted with an end-use product (or with an end-use product whose properties are like those of products to which the determination under paragraph (a) of this section applies). An "end-use product" may be the applicant's own product or a typical end-use product.

(2) Test conditions. The test conditions for conducting a simulated pen, or actual field test should resemble the conditions likely to be encountered under actual use of the product. Specifically, the pesticide should be applied to the site at the rate, frequency, and method specified on the label.

(3) Endangered species. Studies should not be conducted in critical habitats or areas containing, or suspected to contain, endangered or threatened plants or animals which may be threatened by the tests to be conducted.

(4) Residue levels. When the test substance is applied under simulated or actual field condition testing, and residues of the test substance can be detected in warm-blooded animals as evidenced by tests required by 40 CFR Part 158, residues should be determined in selected tissues of test organisms and in vegetation, soil, water, sediments, and other appropriate environmental components. If residues of the test substance cannot be detected in warm-blooded animals as evidenced by tests required by 40 CFR Part 158, then the applicant should consult with the Agency before beginning this test.

(5) Other standards. Additional standards for conducting actual field tests are not delineated because of the wide variety of mechanisms by which a pesticide may enter the environment, and because of the great variety of food sources and habitats that may be affected. Any additional standards for conducting these tests will be provided by the Agency in writing following consultation between the applicant or registrant and the Agency, and will take into account the variety of mechanisms, food sources, and habitats mentioned above.

(c) Reporting and evaluation of data. In addition to the information provided in § 70-4, the test report should contain the following information:

(1) Simulated (pen) field tests.

(i) Test methods and materials data.

(A) Test location;

(B) Dates of beginning and end of test, and any other significant dates of events in the test;

(C) Weather data;

(D) Test species, age and history;

(E) Medical and chemical administration history (if any);

(F) Measured body weights;

(G) Individual identification;

(H) Pesticide chemical formulation, application rate and frequency, and manner of application;

(I) Vegetative cover;

(J) Measured residues in selected tissues of test organisms and in vegetation, soil, water, and sediments, when required; and

(K) Analytical methods for residue determinations;

(L) Housing conditions, including pen description, pen placement and number of animals per pen;

(M) Diet;

(N) Food and water supply schedule;

(O) Feed consumption;

(P) Visual signs of intoxication;

(Q) Clinical measurements for intoxication;

(R) Accidental death or injuries;

(S) Replacement schedule; and

(T) Statistical methods used.

(ii) Test results.

(A) Mortality: number, dates, and other pertinent information;

(B) Toxic signs;

(C) Body weight changes;

(D) Food consumption data;

(E) Clinical observations;

(F) Results of gross necropsy or pathological examinations, if conducted;

(G) Residue analysis results; and

(H) Any noted effects on reproduction.

(iii) Summary and conclusion. Potential hazards to wildlife should be identified in addition to the toxicity results per se.

(2) Actual field tests.

(i) Test methods and materials data.

(A) Description of the study area including vegetation, topography, and all pertinent ecological information;

(B) Study plot layout - locations and replications;

(C) Listing of resident and migrant fauna with estimates of population densities or relative abundance;

(D) Details of application equipment, methods, and weather conditions;

(E) Statistical design and methods of analysis;

(F) Weather data collection methods;

(G) Analytical methods for residue determination;

(H) Clinical data methods;

(I) Reproductive study methods;

(J) Carcass search methods; and

(K) Any other methods utilized.

(ii) Test results.

(A) Mortality: number, dates, and other pertinent information;

(B) Signs of intoxication;

(C) Bird and mammal census results;

(D) Arthropod numbers and biomass;

(E) Food habits data, if any;

(F) Necropsy observations;

(G) Residue analysis results;

(H) Weather data;

(I) Inclusive dates of test; and

(J) Results of nest studies and fledgling wildlife.

(iii) Summary and conclusion. All data should be integrated in a way which reflects the full impact of the pesticide on the ecosystem. Potential hazards to wildlife should be estimated with full consideration of possible indirect effects due to ecological disturbances.

(d) Acceptable protocols.

(1) Simulated (pen) field tests. The following is an example of an acceptable protocol for conducting a simulated (pen) field study for birds. This study is a modification of a study that appears on pages 65 to 73 in an unpublished draft report to EPA from the American Institute of Biological Sciences (AIBS), titled Analysis of Specialized Pesticide Problems, Volume VI, Wildlife Toxicology Study. This report is dated October, 1974 and was funded under EPA contract No. 68-01-2457.

Introduction

For assessing the hazards of pesticides to bobwhite quail, large cages enclosing portions of habitat of 0.005 ha (500 sq ft) or more may be utilized. With modifications other species can also be tested by this method. Tests utilizing large pens may be conducted for pesticides to be applied on cropland, rangeland, or wildlands, or for other outdoor applications such as roadsides, rights-of-way, "waste areas," or known wildlife habitats. This type of test is not a substitute for an actual field study. However, a carefully designed simulated (pen) field study serves to bridge the gap between lab studies and a comprehensive field study.

Methods and Materials

Pens. Wire-covered pens should be constructed covering a minimum ground area of 0.005 ha (500 ft^2 per pen). Suitable minimum pen dimensions for quail are 3.1 m by 15.2 m (10 ft x 50 ft) with the top cover at a height of about 2.0 m (6.5 ft). Other dimensions covering more than 0.005 ha are encouraged. Larger pens will be needed for testing larger birds such as mallards or pheasants.

For uncovered pens, birds may be wing clipped or pinioned. When cage covers are used, they should not interfere with the pesticide reaching the interior of the cage. To reduce predation, metal flashing should be placed around all pens both above and below the ground. In addition, the top perimeter of the fence may be electrified.

A minimum of 3 (10' x 50') pens should be used for each treatment group and control. Each pen should contain 8 pair of quail. Thus a study consisting of one treatment and one control group would have a total of six cages and 96 birds (48 pair). An independent water supply and a small shelter should be furnished in each pen.

Before pens are planned and constructed, wildlife agencies and successful game farms should be consulted to consider factors such as prevention of parasites and disease, soil drainage requirements, support of top cover to prevent collapse under the weight of snow, types of watering equipment, and similar information.

Birds. Adult birds of known history, not previously exposed to pesticides, should be used and placed in the pens at least 2 weeks prior to the pesticide application(s). A supply of replacement bobwhite should be maintained in outdoor pens near the test pens.

Test conditions and procedures. Uniformity of soil type and field conditions are important considerations in selecting study sites. All pens should be numbered and locations mapped or charted. Daily recoreds should be kept of observations, toxic signs, test bird deaths and replacements (if any), complete data on pesticide formulations, application rates and methods, weather conditions, and all other data of value in assessing the hazard to birds. It may be desirable to use moveable pens that can be set up over the crop or vegetation on which the pesticide is intended to be applied. When permanent (non-portable) pens are used, then the soil should be suitable for growing the pertinent crop or vegetation. The pesticide should be applied with the same equipment, at the same rate, timing, number of applications, and formulations as specified on the pesticide label. Additional treatment groups can be added to test the effects of different application techniques (e.g., broadcast versus soil incorporation), application timing (e.g., pre-plant versus postemergent), or irrigation versus nonirrigation. Spraying should be done under minimum wind conditions and with protective shielding to prevent contamination of adjacent sprayed pens or control pens. For statistical purposes, it is best to randomize the test pens, but because of the drift problem, it may be best to stratify the treatment pen locations.

If supplemental feed is needed, the treatment rates can be based on results of residue studies when such studies are available. Where possible, supplemental food should be withheld 1 to 2 days after pesticide treatment. Treated food should be prepared within 1 day of the time the pen environments are sprayed.

The duration of the test should be a minimum of 21 days after the final application, and longer if any birds are showing toxic signs or other effects.

Pesticide residue determinations may be included in the study. Diet and water levels of the test chemicals should be analyzed. Vegetation, soil, and other environmental samples may be analyzed for residues to determine persistence and bioaccumulation. Test birds poisoned by the pesticide and a sample of surviving birds should be analyzed for residues in selected tissues. Gross pathology may be determined at the same time.

(2) Actual field studies for hazard to wildlife. The objective of the actual field study is to determine the impact of pesticide applications on wildlife populations under real-use conditions. Effects of greatest importance include:

- Direct poisoning and death (by ingestion, dermal exposure, or inhalation);
- Toxic effects indirectly causing death such as increasing susceptibility to predation and diseases;
- Harmful reproductive effects and consequent inability to maintain populations.

Full-scale field studies are useful for determining the effects of a pesticide from large-scale spraying of forests, rangelands, rights-of-way, roadsides, wetlands, and major croplands such as cotton, wheat, corn, soybeans, rice, sorghum, or alfalfa, or other major wildlife habitats. In view of the diversity of potential study areas, actual field studies need to be designed on a case-by-case basis. No standard protocol would be appropriate. Therefore, the references that appear in § 71-5(e)(2) can be used as guidance for developing acceptable actual field studies.

(e) References. The following references can provide useful background information for developing acceptable field tests. Some outline useful statistical procedures for handling data.

(1) Simulated (pen) field tests.

(i) Fink, R.J. 1979. Simulated field studies - Acute hazard assessment, Avian and Mammalian Wildlife Toxicology, ASTM STP 693, E.E. Kenaga, Ed., American Society for Testing and Materials, pp. 45-51.

(ii) Kreitzer, J.F., and J.W. Spann. 1968. Mortality among bobwhites confined to a heptachlor contaminated environment. J. Wildl. Manage. 32(4): 874-878.

(iii) Schemnitz, S.D., 1981. Wildlife Management Techniques. 4th Ed.: revised. The Wildlife Society, Inc., Washington, D.C. 722 pp.

(2) Full-scale field tests.

(i) Bart, 1979. Effects of acephate and sevin on forest birds. J. Wild. Manage. 43(2): 544-549.

(ii) Bunyan, P.J., M.J. Van Den Heuvel, P.I. Stanley and E.N. Wright. 1981. An intensive field trial and a multi-site surveillance exercise on the use of aldicarb to investigate methods for the

assessment of possible environmental hazards presented by new pesticides. Agro Ecosystems 7:239-262.

(iii) Caughley, G. 1977. Analysis of Vertebrate Populations. John Wiley and Sons Ltd., A Wiley-Interscience Publication pp. 234.

(iv) De Weese, L.R., C.J. Henny, R.L. Floyd, K.A. Bobal, A.W. Schultz. 1979. Response of breeding birds to aerial sprays of trichlorfon (Dylox) and carbaryl (Seven-4-oil) in forests. Special Scientific Report Wildlife No. 224, U.S. Department of the Interior, Fish and Wildlife Service, Washington, D.C., pp. 29.

(v) Edwards, P.J., S.M. Brown, M.R. Fletcher and P.I. Stanley. 1979. The use of a bird territory mapping method for detecting mortality following pesticide application. Agro Ecosystems 5:271-282.

(vi) Flickinger, E.L., K.A. King, W.F. Stout and M.M. Mohn. 1980. Wildlife hazards from Furadan 3G applications to rice in Texas. J. Wild Manage. 44(1):190-197.

(vii) Hegdal, P.L., T.A. Gatz, K.A. Fagerstone, J.F. Glahn and G.H. Matschke. 1979. Hazards to wildlife associated with 1080 baiting for California ground squirrels. USFWS, under agency Agreement between EPA and FWS, EPA-IAG-D7-0449 (Unpublished final report).

(viii) Hegdal, P.L. T.A. Gatz. 1977. Hazards to pheasants and cottontail rabbits associated with zinc phosphide baiting for microtine rodents in orchards. USFWS, under Interagency Agreement between EPA and FWS, EPA-IAG-D4-0449 (Unpublished final report).

(ix) Herman, S.G., J.B. Bulger. 1979. Effects of a forest application of DDT on nontarget organisms. Wildlife Monographs, No.69, pp. 62.

(x) Johnson, E.V., G.L. Mack and D.Q. Thompson. 1976. The effects of orchard pesticide applications on breeding robins. The Wilson Bull. 88(1):16-35.

(xi) Ludke, J.L., E.F. Hill and M.P. Dieter. 1975. Cholinesterase (ChE) response and related mortality amo fed ChE inhibitors. Arch. Environ. Contam. Toxicol. 3(1):1-21.

(xii) McEwen, L.C., C.E. Knittle, and M.L. Richmond. 1972. Wildlife effects from grasshopper insecticides sprayed on short-grass range. J. Range Manage. 25(3):188-194.

(xiii) Moulding, J.D. 1976. Effects of a low-persistence insecticide on forest bird populations. Auk 93 (4): 692-708.

(xiv) Pearce, P.A., D.B. Peakall and A.J. Erskine. 1976. Impact on forest birds of the 1975 spruce budworm spray operation in New Brunswick. Progress Notes, Canadian Wildlife Service, No. 62, pp 7.

(xv) Ralph, C.J. and J.M. Scott. Eds. 1981. Estimating numbers of terrestrial birds. Studies in Avian Biology No. 6, Cooper Ornithological Society, Pp. 630.

(xvi) Schemnitz, S.D., ed. 1971. Wildlife Managemnt Techiques. 3rd Ed.: revised. The Wildlife Society, Inc., Washington, D.C. 722 pp.

Series 72: AQUATIC ORGANISM TESTING

§ 72-1 Acute toxicity test for freshwater fish.

(a) When required. (1) Data on the acute toxicity of a pesticide to freshwater fish are required by 40 CFR § 158.145 to support the registration of an end-use product intended for outdoor application, and to support each application for registration of a manufacturing-use product which may be used to make such an end-use product.

(2) Data on the acute toxicity of a pesticide to freshwater fish are required by 40 CFR § 158.145, on a case-by-case basis to support the registration of an end-use product intended solely for indoor application, and to support each application for registration of a manufacturing-use product which may be used to make such an end-use product.

(3) See 40 CFR § 158.50, "Formulators' exemption," to determine whether these data must be submitted. Section II-A of this Subdivision provides an additional discussion on this subject.

(b) Test standards. Data sufficient to satisfy the requirements in 40 CFR § 158.145 should be derived from tests which comply with the general test standards in § 70-3 and the following test standards:

(1) Test substance. (i) Data shall be derived from testing conducted with the technical grade of each active ingredient in the product.

(ii) In addition, data from testing with the applicant's end-use product or a typical end-use product are required by 40 CFR § 158.145 to support the registration of products which meet any of the following conditions:

(A) The end-use pesticide will be introduced directly into an aquatic environment when used as directed;

(B) The LC50 or EC50 of the techical grade of active ingredient is equal to or less than the maximum expected environmental concentration (MEEC) or the estimated environmental concentration (EEC) in the aquatic environment when the end-use pesticide is used as directed; or

(C) An ingredient in the end-use product other than the active ingredient is expected to enhance the toxicity of the active ingredient or to cause toxicity to aquatic organisms.

(2) Test organisms. (i) Testing should be performed on one coldwater fish species, preferably rainbow trout, and one warmwater species, preferably bluegill sunfish.

(ii) Very young (not yet actively feeding), spawning, or recently spent fish should not be used.

(iii) Fish should weigh between 0.5 and 5.0 grams and be from the same year class. The standard length of the largest fish should be no more than twice that of the shortest fish.

(3) Determination of LC50. (i) Satisfactory data must establish either:

(A) A 96-hour LC50 value with 95 percent confidence intervals; or

(B) That the 96-hour LC50 is greater than 100 mg/l or greater than 100,000 times the MEEC or EEC.

(ii) If data are submitted to satisfy either criterion in paragraph (b)(3)(i)(B) of this section, at least 30 individuals should be tested at concentrations equal to or greater than the criterion chosen.

(c) Reporting and evaluation of data. In addition to information provided in § 70-4, a report of the results of a fish acute LC50 test should include the following:

(1) LC50 data. (i) (A) Data showing the 96-hour LC50, the corresponding 95 percent confidence intervals, slope of the concentration response line, and, when possible, the LC50 values at 24, 48, and 72 hours; or

(B) Data showing that the 96-hour LC50 is greater than 100,000 times the MEEC or EEC or greater than 100 mg/l.

(ii) If the data submitted in accordance with paragraph (c)(1)(i)(B) of this section show that the LC50 is greater than 100,000 times the MEEC or EEC of the pesticide; the basis for calculating the MEEC or EEC should be reported.

(2) Dilution water. Detailed description of dilution water, including source, chemical characteristics (e.g., dissolved oxygen content, pH, dissolved salts), and pretreatment (if any).

(3) Test description. Detailed description of the test, including:

(i) Design;

(ii) Containers;

(iii) Water depth and volume;

(iv) Treatments;

(v) Method of exposing fish to the test substance (e.g., placing chemical in water which already contains fish or placing fish in water which already contains the chemical);

(vi) Number of organisms per treatment;

(vii) Loading (weight of organisms per unit volume of water);

(viii) Lighting, acclimation, and test temperatures (averages and range); and

(ix) Any unusual feature of the test methodology.

(4) Chemical analyses. If conducted, a description of the methods (or references to established methods) used for all the analyses of water for chemical content and toxicant concentrations, and the results of such analyses, including validation studies and reagent blanks.

(5) Effects of exposure. Detailed description of the effects of exposure to the test substance including:

(i) The criteria used to determine the effects;

(ii) Percentages of organisms that died or showed effects of treatment; and

(iii) A summary of these observations.

(6) Additional information. Any additional relevant information about the test or its results that would assist in the determination of hazard potential.

(d) Acceptable protocols. Examples of acceptable protocols for conducting a freshwater fish acute toxicity study are found in the following references. Fish species listed in these publications are acceptable with the exception of goldfish.

(1) ASTM Standard E 729-80, Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

(2) Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. U.S. Environmental Protection Agency, Ecol. Res. Series, EPA 660/3-75-009. 61 pp.

(e) References. The following references can provide useful background information in developing acceptable protocols:

(1) Weber, C. E. (ed.) 1973. Biological field and laboratory methods for measuring the quality of surface waters and effluents. U.S. Environmental Protection Agency, Environ. Monit. Series, EPA-670/4-73-001.

(2) Anonymous. 1981. Standard Methods for the Examination of Water and Wastewater. 15th Ed. American Public Health Assoc., Washington, D.C. 1134 pp.

§ 72-2 Acute toxicity test for freshwater aquatic invertebrates.

(a) When required. (1) Data on the acute toxicity of a pesticide to freshwater aquatic invertebrates are required by 40 CFR § 158.145 to support the registration of an end-use product intended for outdoor application, and to support each application for registration of a manufacturing-use product which may be used to make such an end-use product.

(2) Data on the acute toxicity of a pesticide to freshwater aquatic invertebrates are required by 40 CFR § 158.145, on a case-by-case basis to support the registration of an end-use product intended solely for indoor application, and to support each application for registration of a manufacturing-use product which may be used to make such an end-use product.

(3) See 40 CFR § 158.50, "Formulators' exemption," to determine whether these data must be submitted. Section II-A of this Subdivision provides additional discussion on this subject.

(b) Test standards. Data sufficient to satisfy the requirements in 40 CFR § 158.145 should be derived from tests which comply with the general test standards in § 70-3 and the following test standards.

(1) Test substance. (i) Data shall be derived from testing conducted with the technical grade of each active ingredient in the product.

(ii) In addition, data from testing with the applicant's end-use product or a typical end-use product are required by 40 CFR § 158.145 to support the registration of products which meet any of the following conditions:

(A) The end-use product will be introduced directly into an aquatic environment when used as directed;

(B) The LC50 or EC50 of the technical grade of active ingredient is equal to or less than the maximum expected environmental concentration (MEEC) or the estimated environmental concentration (EEC) in the aquatic environment when the end-use product is used as directed; or

(C) An ingredient in the end-use product other than the active ingredient is expected to enhance the toxicity of the active ingredient or to cause toxicity to aquatic organisms.

(2) Test organisms. Immature invertebrates should be used whenever possible. Among fresh-water organisms, daphnids should be in the first instar; amphipods, stoneflies, and mayflies in an early instar; and midges in the second or third instar.

(3) Determination of EC50 or LC50. (i) Satisfactory data should establish either:

(A) An EC50 or LC50 value with 95 percent confidence intervals; or

(B) That the EC50 or LC50 is greater than 100 mg/l or greater than 100,000 times the MEEC or EEC.

(ii) If data are submitted to satisfy either criterion in paragraph (b)(3)(i)(B) of this section, at least 30 individuals should be tested at concentrations equal to or greater than the criterion chosen.

(4) Duration of tests. Daphnids and midge larvae should be exposed to the test substance for 48 hours in static tests. All other organisms should be exposed for 96 hours in static tests. For flow-through tests, all organisms tested under this section should be exposed for at least 96 hours.

(c) Reporting and evaluation of data. In addition to information provided in § 70-4, a report of the results of an acute toxicity test for aquatic invertebrates should include the following:

(1) LC50 data. (i) (A) Data showing the EC50 or LC50, the corresponding 95 percent confidence intervals, slope of the concentration reponse line, and when possible, the EC50 or LC50 values at 24-hour intervals for the duration of the test; or

(B) Data showing that the EC50 or LC50 is greater than 100,000 times the MEEC or EEC or greater than 100 mg/l.

(ii) If the data submitted in accordance with paragraph (c)(1)-(i)(B) of this section show that the LC50 or EC50 is greater than 100,000 times the MEEC or EEC of the pesticide, the basis for calculating the MEEC or EEC should be reported.

(2) Dilution water. Detailed description of dilution water, including source, chemical characteristics (e.g., dissolved oxygen content, pH, dissolved salts), and pretreatment (if any).

(3) Test description. Detailed description of the test, including:

(i) Design;

(ii) Containers;

(iii) Water depth and volume;

(iv) Treatments;

(v) Method of exposing organisms to the test substance (e.g., placing chemical in water which contains organisms or placing organisms in water which contains chemical);

(vi) Number of organisms per treatment;

(vii) Lighting, acclimation, and test temperatures (averages and range); and

(viii) Any unusual feature of the test methodology.

(4) Chemical analyses. If conducted, a description of the methods (or references to established methods) used for the analyses of water for chemical content and toxicant concentrations, and the results of such analyses, including validation studies and reagent blanks.

(5) Effects of exposure. Detailed description of the effects of exposure to the test substance, including:

(i) The criteria used to determine the effects;

(ii) Statement of percentages of organisms that died or showed effects of treatment; and

(iii) A summary of these observations.

(6) Additional information. Any additional relevant information about the test or its results that would assist in the determination of hazard potential.

(d) Acceptable protocols. Examples of acceptable protocols for conducting a freshwater aquatic invertebrate acute toxicity study are found in the following references:

(1) ASTM Standard E 729-80, Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. American Society for Testing and Materials, 1916 Race Street, Philadephia, Pa. 19103.

(2) Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. U.S. Environmental Protection Agency, Ecol. Res. Series, EPA 660/375-009. 61 pp. (Aquatic invertebrate test temperatures in this publication are acceptable with the exception of 17°C for Daphnia spp. Daphnia should be tested at 20°+ 1°C.)

(e) Reference. The following publication can provide useful background information in developing acceptable protocols:

Anonymous. 1981. Standard Methods for the Examination of Water and Wastewater. 15th Ed. American Public Health Assoc., Washington, D.C. 1134 pp.

§ 72-3 Acute toxicity test for estuarine and marine organisms.

(a) When required. (1) Data on the acute toxicity of a pesticide to estuarine and marine organisms are required by 40 CFR § 158.145 to support the registration of an end-use product intended for direct application to the estuarine or marine environment or

expected to enter this environment in significant concentrations because of its expected use or mobility pattern.

(2) See 40 CFR § 158.50, "Formulators' exemption," to determine whether these data must be submitted. Section II-A of this Subdivision provides an additional discussion on this subject.

(b) Test standards. Data sufficient to satisfy the requirements in 40 CFR § 158.145 should be derived from tests which comply with the general test standards in § 70-3 and the following test standards:

(1) Test substance. (i) Data shall be derived from testing conducted with the technical grade of each active ingredient in the product.

(ii) In addition, data from testing with the applicant's end-use product or a typical end-use product are required by 40 CFR § 158.145 to support the registration of products which meet any of the following conditions:

(A) The end-use product will be introduced directly into an aquatic environment when used as directed;

(B) The EC50 or LC50 of the technical grade of active ingredient is equal to or less than the maximum expected environmental concentration (MEEC) or the estimated environmental concentration (EEC) in the aquatic environment when the end-use product is used as directed; or

(C) An ingredient of the end-use product other than the active ingredient is expected to enhance the toxicity of the active ingredient or to cause toxicity to aquatic organisms.

(2) Test organisms and test duration. The 96-hour LC50 should be determined for shrimp and an estuarine or marine fish. Also, the 48-hour EC50 for oyster embryolarvae or 96-hour EC50 shell deposition data should be determined on a representative mollusc, such as the American oyster.

(3) Determination of EC50 or LC50. (i) Satisfactory data should establish either:

(A) An EC50 or LC50 value with 95 percent confidence intervals; or

(B) That the EC50 or LC50 is greater than 100 mg/l or greater than 100,000 times the MEEC or EEC.

(ii) If data are submitted to satisfy either criterion in paragraph b)(3)(i) of this section, at least 30 individuals should be tested at concentrations equal to or greater than the criterion chosen.

(c) Reporting and evaluation of data. In addition to information provided in § 70-4, a report of the results of an acute toxicity test for estuarine and marine organisms should include the following:

(1) LC50 data. (i) (A) Data showing the LC50 or EC50, the corresponding 95 percent confidence intervals, slope of the concentration-response line, and, when possible, the LC50 values at 24-hour intervals for the duration of the test; or

(B) Data showing that the LC50 or EC50 is greater than 100,000 times the MEEC or EEC or greater than 100 mg/l.

(ii) If the data submitted in accordance with paragraph (c)(1)-(i)(B) of this section show that the LC50 or EC50 is greater than 100,000 times the MEEC or EEC of the pesticide, the basis for calculating the MEEC or EEC should be reported.

(2) Dilution water. Detailed description of dilution water, including source, chemical characteristics (e.g., dissolved oxygen content, pH), and pretreatment (if any).

(3) Test description. Detailed description of the test, including:

(i) Design;

(ii) Containers;

(iii) Water depth and volume;

(iv) Treatments;

(v) Method of exposing organisms to the test substance (e.g., placing chemical in water which contains organisms or placing organisms in water which contains the chemical);

(vi) Number of organisms per treatment;

(vii) Loading (weight of organisms per unit volume of water);

(viii) Lighting;

(ix) Acclimation and test temperatures (average and range);

(x) Salinities; and

(xi) Any unusual feature of the test.

(4) Chemical analyses. If conducted, a description of methods (or references to established methods) used for the analyses of water for chemical content and toxicant concentrations, and the results of such analyses, including validation studies and reagent blanks.

(5) Effects of exposure. Detailed description of the effects of the exposure to the test substance, including:

(i) The criteria used to determine the effects;

(ii) Statement of percentages of organisms that died or showed effects of treatment; and

(iii) A summary of these observations.

(6) Additional information. Any additional relevant information about the test or its results that would assist in the determination of hazard potential.

(d) Acceptable protocols.

(1) Acute estuarine and marine fish, and shrimp toxicity tests. Examples of acceptable protocols are found in the following references:

(i) Bahner, L.H., C.D. Craft, and D.R. Nimmo. 1975. A salt-water flow-through bioassay method with controlled temperature and salinity. Prog. Fish-Cult. 37(3):126-129.

(ii) Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. U.S. Environmental Protection Agency, Ecol. Res. Series, EPA 660/375-009. 61 pp. (Marine and estuarine species listed in this publication are acceptable.)

(2) Marine mollusc shell deposition and embryolarvae toxicity tests. Examples of acceptable protocols are found in the following references:

(i) Anonymous. 1981. Standard Methods for the Examination of Water and Wastewater. 15th Ed. American Public Health Association, Washington, D.C. 1134 pp.

(ii) ASTM Standard E 724-80, Practice for Conducting Static Acute Toxicity Tests with Larvae of Four Species of Bivalve Molluscs. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

(iii) Woelke, C.E. 1967. Measurement of water quality with the Pacific oyster embryo bioassay. Pp. 112-120 in Water Quality Criteria, ASTM, STP 416. American Society for Testing and Materials, Philadelphia, Pa.

(e) References. The following references can provide useful background information in developing acceptable protocols:

(1) Anonymous. 1978. Bioassay Procedures for the Ocean Disposal Permit Program. U.S. Environmental Protection Agency, Office of Res. and Dev. EPA-600/9-78-010. 121 pp.

(2) Clark, J.R., and R.L. Clark, eds. 1964. Seawater systems for experimental aquariums. U.S. Dept. Int., Fish. & Wild. Serv. Bur. Sport. Fish. Wild. Res. Rep. #63. 192 pp.

(3) DeBen, E.A. 1970. Design and construction of saltwater environment simulator. Fed. Water Qual. Admin., Pacific N.W. Water Lab., Working Paper 71:1-30.

(4) Strickland, J.D.H., and T.R. Parsons. 1968. A practical handbook of seawater analysis. Fish. Res. Board Can. Bull. No. 167. 311 pp.

(5) White, D.B., R.R. Stickney, D. Miller, and L.H. Knight. 1973. Seawater systems for aquaculture of estuarine organisms at the Skidaway Institute of Oceanography. Ga. Mar. Sci. Center, Technical Rep. Ser. No. 73-1. 18 pp.

(6) Wood, L. 1975. A controlled condition system (CCS) for continuously flowing seawater. Limnol. Oceanogr. 10:475-477.

§ 72-4 Fish early life-stage and aquatic invertebrate life-cycle studies.

(a) When required. (1) Data from fish early life-stage tests or lifecycle tests with aquatic invertebrates (whichever species is most sensitive to the pesticide as determined from the results of tests performed in §§ 72-1, -2, and -3) are required by 40 CFR § 158.145 to support the registration of an end-use product intended to be applied directly to water or expected to be transported to water from the intended use site, and when any of following conditions apply:

(i) If the pesticide is intended for use such that its presence in water is likely to be continuous or recurrent regardless of toxicity, as revealed by studies required by 40 CFR § 158.130; or

(ii) If any LC50 or EC50 value determined in testing required by §§ 72-1, -2, or -3 is less than 1 mg/l; or

(iii) If the estimated environmental concentration in water is equal to or greater than 0.01 of any EC50 or LC50 determined in acute testing for aquatic organisms required by 40 CFR § 158.145; or

(iv) If the actual or estimated environmental concentration in water resulting from use is less than 0.01 of any EC50 or LC50 determined in acute testing for aquatic organisms required by 40 CFR § 158.145 and any of the following conditions exists:

(A) Studies of other organisms indicate the reproductive physiology of fish and/or invertebrates may be affected; or

(B) Physiochemical properties indicate cumulative effects; or

(C) The pesticide is persistent in water (e.g., half-life in water greater than 4 days).

(2) See 40 CFR § 158.50, "Formulators' exemption," to determine whether these data must be submitted. Section II-A of this Subdivision provides an additional discussion on this subject.

(b) Test standards. Data sufficient to satisfy the requirements in 40 CFR § 158.145 should be derived from tests which comply with the general test standards in § 70-3 and the following test standards:

(1) Test substance. Data shall be derived from testing conducted with the technical grade of each active ingredient in the product.

(2) Duration of tests.

(i) Fish early life-stage test. Fish should be exposed to the test substance through the embryolarvae phase (e.g., a fish "egg-fry" test) but not all stages of life-cycle of one generation of the species.

(ii) Invertebrate life-cycle test. Invertebrates should be cultured in the presence of the test substance from one stage of its life-cycle to at least the same stage of the next generation (e.g., egg to egg).

(3) Species. The applicant should consult with the Agency regarding the appropriate species and test methodologies. The choice of species and test methods will be tailored to the pesticide's characteristics.

(4) Concentration analysis. The concentration of the test substance in the water should be determined at the start of the study, and periodically throughout the study to verify concentrations.

(c) Reporting and evaluation of data. In addition to the basic information provided in § 70-4, the test report should contain the following information (where appropriate):

(1) Reproductive effects;

(2) Detailed records of spawning, egg numbers, fertility, and fecundity;

(3) No effect level;

(4) Mortality data;

(5) Statistical evaluation of effects;

(6) Locomotion, behavioral, physiological, and pathological effects;

(7) Definition of the criteria used to determine effects;

(8) Summary of general observation of signs of intoxication or other effects;

(9) Stage of life cycle in which organisms were tested;

(10) Duration of the test; and

(11) Concentration analysis.

(d) Acceptable protocols.

(1) Fish early life-stage test. Examples of acceptable protocols are found in the following references:

(i) National Water Quality Laboratory Committee on Aquatic Bioassays. 1971. Recommended bioassay procedure for fathead minnow Pimephales promelas (Rafinesque) chronic tests. (Revised January, 1972). Pp. 15-24 in Biological Field and Laboratory Methods. U. S. Environmental Protection Agency, Office of Res. and Dev. EPA-670/473-001.

(ii) _____ 1971. Recommended bioassay procedure for brook trout Salvelinus fontinalis (Mitchell) partial chronic tests. (Revised January, 1972). Pp. 25-33 in Biological Field and Laboratory Methods. U.S. Environmental Protection Agency, Office of Res. and Dev. EPA-670/4-73-001.

(2) Invertebrate life-cycle test. Examples of acceptable protocols are found in the following references:

(i) Biesinger, K.E. 1974(a). Procedure for Daphnia magna chronic tests in standing system. U.S. Environmental Protection Agency, Environ. Res. Lab., Duluth, Minnesota. Fed. Regis. 40(123):26902-26903. (June 25, 1975.)

(ii) Biesinger, K.E. 1974(b). Procedure for Daphnia magna chronic tests in flowing system. U.S. Environmental Protection Agency, Environ. Res. Lab., Duluth, Minnesota. Fed. Regis. 40(123): 26903. (June 25, 1975.)

(iii) Nimmo, D.E., T.L. Hamaker, and C.A. Sommers. 1978. Entire life-cycle toxicity test using mysids (Mysidopsis bahia) in flowing water. Pp. 64-68 in Bioassay Procedures for the Ocean Disposal Permit Program. U.S. Environmental Protection Agency, Office of Res. and Dev. EPA-600/9-78-010.

(e) Reference. Additional information may be found in the following reference:

Biesinger, K.E. 1974(c). Culturing methods for Daphnia and certain other cladocerans. U.S. Environmental Protection Agency, Environ. Res. Lab., Duluth, Minnesota. Fed. Regis. 40(123):26903-26904. (June 25, 1975.)

§ 72-5 Life-cycle tests of fish.

(a) When required. (1) Data obtained from a life-cycle test of fish are required by 40 CFR § 158.145 to support the registration of an end-use product intended to be applied directly to water or expected to transport to water from the intended use site, and when any of the following conditions apply:

(i) If the estimated environmental concentration is equal to or greater than one-tenth of the no-effect level in the fish early life-stage or invertebrate life-cycle test; or

(ii) If studies of other organisms indicate the reproductive physiology of fish may be affected.

(2) See 40 CFR § 158.50, "Formulators' exemption", to determine whether these data must be submitted. Section II-A of this Subdivision provides an additional discussion on this subject.

(b) Test standards. Data sufficient to satisfy the requirements in 40 CFR § 158.145 should be derived from tests which comply with the general test standards in § 70-3 and the following test standards:

(1) Test substance. Data shall be derived from testing conducted with with the technical grade of each active ingredient in the product.

(2) Duration of tests. Fish should be cultured in the presence of the test substance from one stage of the life cycle to at least the same stage of the next generation (e.g., egg to egg).

(3) Species. Testing should be performed on a freshwater fish (e.g., fathead minnow). An estuarine species (e.g., sheepshead minnow) may be used if the pesticide is expected to enter the estuarine environment.

(4) Concentration analysis. The concentration of the test substance in the water should be determined at the start of the study and periodically throughout the study to verify concentrations.

(c) Reporting and evaluation of data. In addition to the basic information provided in § 70-4, the test report should contain the following information (where appropriate):

(1) Reproductive effects;

(2) Detailed records of spawning, egg numbers, fertility, and fecundity;

(3) No-effect level, and mortality data;

(4) Statistical evaluation of effects;

(5) Locomotion, behavioral, physiological, and pathological effects;

(6) Definition of the criteria used to determine effects;

(7) Summary of general observation of signs of intoxication or other effects;

(8) Stage of life cycle in which organisms were tested;

(9) Duration of the test; and

(10) Concentration analysis.

(d) Acceptable protocol.

(1) Freshwater fish life-cycle test. An example of an acceptable protocol is found in the following reference:

National Water Quality Laboratory Committee on Aquatic Bioassays. 1971. Recommended bioassay procedure for fathead minnow Pimephales promelas (Rafinesque) chronic tests. (Revised January, 1972). Pp. 15-24 in Biological Field and Laboratory Methods. U. S. Environmental Protection Agency, Office of Res. and Dev. EPA-670/4-73-001.

(2) Estuarine fish life-cycle test. Examples of acceptable protocols are found in the following references:

(i) Schimmel, S.C., and D.J. Hansen. 1974. Sheepshead minnow Cyprinodon variegatus: an estuarine fish suitable for chronic (entire lifecycle) bioassays. Proc. 28th Ann. Cong. S.E. Assoc. Game-Fish Comm. Pp. 392-398.

(ii) Hansen, D.J., P.R. Parrish, S.C. Schimmel, and L.R. Goodman. 1978. Life-cycle toxicity test using sheepshead minnows (Cyprinodon variegatus). Pp. 109-116 in Bioassay Procedures for the Ocean Disposal Permit Program. U.S. Environmental Protection Agency, Office of Research and Development. EPA-600/9-78-010.

§ 72-6 Aquatic organism accumulation tests.

(a) When required. Data from aquatic organism accumulation testing are required by 40 CFR § 158.145 on a case-by-case basis to support the registration of an end-use product whose use is likely to result in residues in an aquatic environment and which may accumulate in aquatic organisms to toxic levels. Consultation with the Agency is advised before undertaking this test. The determination that a product meets these conditions should be made when any of the following conditions apply:

(1) If the active ingredient or its principal degradation product(s):

(i) Has water solubility less than 0.5 mg/l and an octanol/water partition coefficient greater than 1000; and

(ii) Is persistent in water (e.g., a half-life greater than four days); or

(2) If the active ingredient or its principal degradation product(s) accumulates in the organs and tissues of mammals or avian species.

(b) Test standards. Data sufficient to satisfy the requirements in 40 CFR § 158.145 should be derived from tests which comply with the general test standards in § 70-3 and the following test standards:

(1) Test substance. Data shall be derived from testing conducted with the technical grade of each active ingredient in the product (studies using radioisotopes require analytical grade) or the purest available form of the principal degradation products, whichever meets the general or specific conditions set forth in paragraph (a)(i) and (ii).

(2) Test organisms. (i) Consultation with the Agency is advised before selection of species is made. One or more of the following species may be used in accumulation testing:

(A) A typical bottom-feeding fish (e.g., catfish or carp);

(B) A cold-water fish, a warm-water fish, or marine fish (e.g., brook trout, rainbow trout, bass, bluegill, northern pike, walleye, or sheepshead minnow);

(C) Molluscs (e.g., oyster or freshwater clams);

(D) Crustaceans (e.g., Daphnia spp., shrimp, or crayfish); or

(E) Insect nymphs (e.g., mayfly).

(ii) The following factors should be considered in selecting species:

(A) The use pattern of the formulated product;

(B) The relative sensitivity of the different species to toxic effects; and

(C) Data on route of exposure and method of uptake.

(c) Reporting and evaluation of data. In addition to the information provided in § 70-4, specific data reporting and evaluation guidance should be determined by consultation with the Agency.

(d) References. The following references can provide useful background information in developing protocols. The conditions under which an accelerated aquatic organism test [reference (d)(4)] may be an acceptable substitute for a full length test [references (d)(1)-(3)] should be determined by consulting with the Agency.

(1) Johnson, B.T., and R.A. Schoettger. 1975. A biological model for estimating the uptake, transfer, and degradation of xenobiotics in a food chain. Fed. Regis. 40 (123): 26906-26909. (June 25, 1975.)

(2) Macek, K.J., M.E. Burrows, R.F. Frasny, and B.H. Sleight, III. 1975. Bioconcentration of ^{14}C pesticides by bluegill sunfish during continuous exposure. Pp. 119-142 in Structure-activity correlations in studies of toxicity and bioconcentration with aquatic organisms. Proceedings of a Symposium, Burlington, Ontario, March 11-13, 1975. G.D. Veith and D.E. Konasewich, eds. Sponsored by Standing Committee on Scientific Basis for Water Quality Criteria of the International Joint Commission's Research Advisory Board.

(3) Schimmel, S.C., J.M. Patrick Jr., and A.J. Wilson. 1977. Acute toxicity to and bioconcentration of endosulfan by estuarine animals. Pp. 241-252 in Aquatic Toxicology and Hazard Evaluation. F.L. Mayer and J.L. Hamelink, eds. STP #634, American Society for Testing and Materials, Philadelphia, Pa.

(4) Branson, D.R., G.E. Blau, H.C. Alexander, and W.B. Neely. 1975. Bioconcentration of 2,2',4,4'-tetrachlorobiophenyl in rainbow trout as measured by an accelerated test. Trans. Am. Fish. Soc. 104(4):785-792.

§ 72-7 Simulated or actual field testing for aquatic organisms.

(a) When required. (1) Data from any of the following kinds of tests are required by 40 CFR § 158.145, on a case-by-case basis, to support the registration of an end-use product intended for outdoor application. Consultation with the Agency is advised before undertaking these tests. Whenever data are required by 40 CFR § 158.145, the determination will be made in writing by the Agency and will state which properties and use patterns of the product were used in the determination. The following criteria are provided as further guidance;

(i) Data from a short-term simulated field test (or an actual short-term field test) are required by 40 CFR § 158.145 to support the registration of an end-use product which is likely to cause adverse short-term or acute effects on fish or aquatic invertebrates. The short-term simulated field test (where confined populations are observed) should be selected if it can yield data useful in assessing such risks. An actual short-term field test (where natural populations are observed) may be needed if a simulated test would not suffice. The determination of test selection or whether either should be conducted should take into account available laboratory toxicity data, use pattern information, and exposure information.

(ii) Data from a long-term simulated field test (or an actual long-term field test) are required by 40 CFR § 158.145 to support the registration of an end-use product which is likely to cause adverse long-term, cumulative, or life-cycle effects in fish or aquatic invertebrates. The long-term simulated field test (where growth and reproduction of confined populations are observed) should be selected if it can yield data useful in assessing such risks. An actual long-term field test (where growth and reproduction of natural populations are observed) may be needed if the simulated test would not suffice. The determination of test selection or whether either should be conducted should take into account available laboratory toxicity data, use pattern information, and exposure information.

(2) See 40 CFR § 158.50, "Formulators' exemption," to determine whether these data must be submitted. Section II-A of this Subdivision provides an additional discussion on this subject.

(b) Test standards. Data sufficient to satisfy the requirements in 40 CFR § 158.145 should be derived from tests which comply with the general test standards in § 70-3 and the following test standards:

(1) Test substance. Unless specified otherwise, data shall be derived from testing conducted with an end-use product [or with an end-use product whose properties are like those of products to which the determination under paragraph (a) of this section applies]. An "end-use product" may be the applicant's own product or a typical end-use product.

(2) Concentration analysis. The concentration of the test substance in the water should be determined at the start of the study and collected periodically for analysis to verify concentrations.

(3) Test conditions. The test conditions for conducting field tests should resemble the conditions likely to be encountered under actual use. Specifically, the pesticide should be applied according to the rate, frequency, and method specified on the label.

(4) Endangered species. Studies should not be conducted in critical habitats or areas containing, or suspected to contain, endangered or threatened plants or animals which may be threatened by the tests to be conducted.

(5) Residue levels. When the test substance is applied under simulated or actual field condition testing, residues should be determined in appropriate vegetation, soil, water, sediments, and other environmental components, and in selected tissues of test organisms.

(6) Other standards. Any additional standards for conducting these tests will be provided by the Agency in writing following consultation between the applicant and the Agency, and will take into account the mechanisms by which a pesticide may enter the environment, and the food sources and habitats that may be affected.

(c) Reporting and evaluation of data. In addition to the information provided in § 70-4, specific data reporting and evaluation guidance should be determined by consultation with the Agency.

(d) References. The following references can provide useful background information for conducting a simulated or actual field study for aquatic organisms.

(1) Coppage, D.L. 1971. Characterization of fish brain acetylcholinesterase with an automated pH stat for inhibition studies. Bull. Environ. Contam. Toxicol. 6(4):304-310.

(2) Kingsbury, P.D. 1976. Studies of the impact of aerial applications of the synthetic pyrethroid NRDC-143 on aquatic ecosystems. Chemical Control Research Institute, Department of the Envornment, Ottawa, Ontario. Report CC-X-127 (unpublished report).

(3) Macek, K.J., D.F. Walsh, J.W. Hogan and D.D. Holz. 1972. Toxicity of the insecticide Dursban® to fish and aquatic invertebrates in ponds. Trans. Am. Fish. Soc. 101(3):420-427.

(4) Nicholson, H.P., H.J. Webb, G.J. Lauer, R.E. O'Brian, A.R. Grzenda and D.W. Shanklin. 1962. Insecticide contamination in a farm pond. Part I - Origin and Duration; Part II - Biological Effects. Trans. Am. Fish Soc. 91(2):213222.

(5) Schemnitz, S.D., ed. 1980. Wildlife Management Techniques. 4th Ed.: revised. The Wildlife Society, Inc., Washington, D.C. 722 pp.

(6) Tagatz, M.E., P.W. Borthwick, G.H. Cook and D.L. Coppage. 1974. Effects of ground applications of Malathion on salt marsh environments in northwestern Florida. Mosquito News 34(3):309-315.

(7) U.S. Dept. of Interior. 1977. National Handbook of Recommended Methods for Water Data Acquisition. U.S. Geological Survey, Reston, VA.

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